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En Ecologie et Biodiversité

École doctorale GAIA

Unité de recherche : Centre d'Ecologie Fonctionnelle et Evolutive (CEFE – UMR 5175)

Breeding with seasons: Variability, heritability, and natural selection of phenological plasticity in a wild bird

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Le 22 novembre 2019

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Organisms live in environments that vary over space and time. Species can adapt to environmental variability via different (and non-exclusive) mechanisms, depending on the temporal and/or spatial scales and predictability of environmental variation (Botero et al., 2015; Tufto, 2015). First, organisms can adapt to environmental changes via adaptive microevolution, that is, change in allele frequency through time caused by natural selection. Such microevolution may occur in coarse-grained environments, i.e. when the scale of the environmental variation is much larger than that encountered by individuals during a generation, regardless of environmental predictability. For example, in Trinidadian guppies (*Poecilia reticulata*), males differ in colour, morphology, and behaviour among natural populations in South America. These differences have been shown to be the result of local adaptation to different communities of predators via genetic differentiation (Reznick, 1982; Reznick et al., 1997). Second, when the temporal and/or spatial scales of environmental variation is on the order of a few generations, and environmental changes are unpredictable, a diversifying bet-hedging strategy should be favoured (Botero et al., 2015; Tufto, 2015), so as to ‘spread the risk’ when the environment is unpredictable. A bet-hedging genotype produces diversified offspring phenotypes, adapted to different environmental conditions. Hence, in any encountered environment, some offspring would be able to survive and reproduce among the progeny of such genotype (see e.g. Starrfelt and Kokko, 2012; Childs et al., 2010). One of the most famous examples of bet-hedging is plant adaptation to unpredictable rainfall, where plants produce seeds with variable dormancy times (Cohen, 1966). Finally, when environmental variability is predictable and the temporal and/or spatial scale(s) of the environmental variation is on the order of one generation or less, phenotypic plasticity is selected for (Botero et al., 2015; Tufto, 2015).

1. Phenotypic plasticity

Phenotypic plasticity is the environment-dependent phenotype expression of a given genotype (Pigliucci, 2001; DeWitt and Scheiner, 2004a; Streans, 1989). The concept of phenotypic plasticity has a long scientific history (Woltereck, 1909), but it has received renewed interest in the scientific community in recent decades (figure 1). Plasticity is ubiquitous in nature (Travis, 1994; Pigliucci, 2001; DeWitt and Scheiner, 2004a) and encompasses a large range of biological phenomena such as: i) alternative wings colour patterns depending on the developmental season in the ‘map butterfly’ *Araschnia Levana* (figure 2, Friberg and Karlsson, 2010; ii) worker caste differentiation according to rearing condition in the ant *Pheidologeton diversus* (figure 3, Holldobler and Wilson, 1990); iii) darkening of the skin in humans according to sunlight exposure or iv) change in colour, shape and behaviour of the Octopus *cyanea mollusca* depending on the local environment (figure 4, Josef et al., 2012). While the two former examples are developmental plasticity, that is change in developmental pathways depending on the environment leading to irreversible phenotypes, the two latter examples are cases of labile plasticity. Labile plasticity is the re-

versible change of a phenotype depending on environment (i.e. the phenotype tracks environmental changes several times during an individual's lifetime). When the temporal scale of the environmental variation is close to generation time, irreversible developmental phenotypic plasticity is favoured by natural selection. On the contrary, environmental variation on a shorter scale than generation time will promote evolution of reversible labile plasticity (Botero et al., 2015; Tufto, 2015; Gabriel et al., 2005).

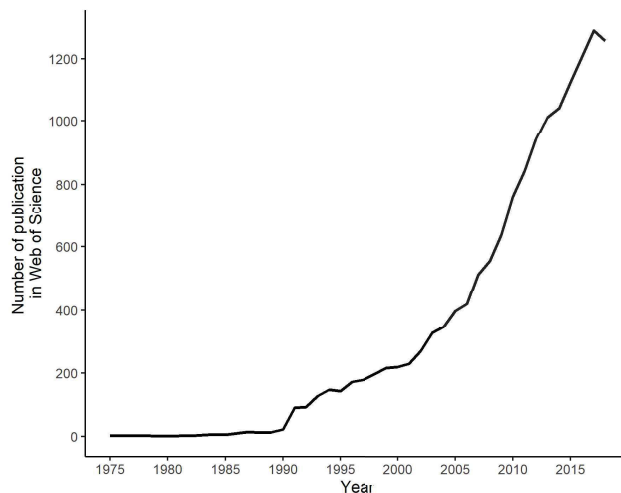


Figure 1: Increasing number of scientific publications through years associated with the keywords ‘phenotypic plasticity’ in the article research motors ‘Web of Science’.

Phenotypic plasticity can evolve when natural selection on a trait varies as a function of predictable environmental conditions (sometimes named ‘informational plasticity’, e.g. Nettle and Bateson, 2015). The environment then acts as a cue that provides information about future selective pressures on the (plastic) phenotype (Nettle and Bateson, 2015), allowing organisms to modulate their phenotype so as to match selective pressures in future environmental conditions. More formally, consider for simplicity a binary trait in a binary environment (such as a insect polytheism over different host plants for example), the concept of phenotypic plasticity of a trait Z according to an environmental cue E in environmental conditions e can be summarized as (Nettle and Bateson, 2015):

$$p(e|E) > p(e|\neg E) \quad (1)$$

$$w(Z|e) > w(\neg Z|e) \text{ and } w(\neg Z|\neg e) > w(Z|\neg e) \quad (2)$$

$$\text{Because of (1) and (2), } w(Z|E) > w(\neg Z|E) \text{ and } w(\neg Z|\neg E) > w(Z|\neg E) \quad (3)$$

with \neg meaning ‘not’ (e.g. $\neg E$ means ‘no environmental cue’), p the probability of an event and w the fitness associated to a given phenotype. This formalism

can be extended to continuous phenotypes (such as phenology) and continuous environments (such as temperature).

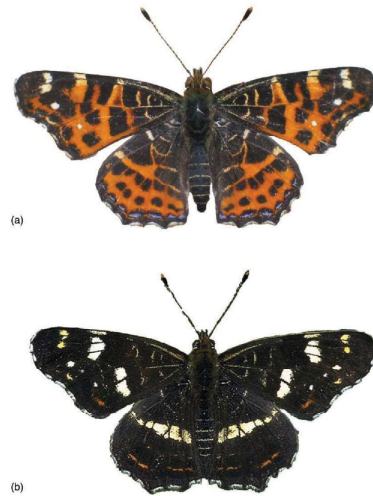


Figure 2: Spring a) and winter b) wings color patterns in *Araschnia Levana*, the ‘map butterfly’. Credits: Friberg and Karlsson (2010).

One of the most famous examples of plasticity is the response to the presence of predators in *Daphnia*. For example, in *Daphnia lumholtzi*, a given genotype can produce individuals with a round head shape and a small tail in the absence of predator, and individuals with a peaked helmet and a long spine tail when exposed to chemical cues (or kairomones) indicating the presence of predators (figure 5, see e.g. Agrawal, 2001). This occurs because the probability that predators are present is higher conditional on the detection of kairomones than in their absence (equation 1); the ‘helmet phenotype’ is fitter than the ‘round head phenotype’ in the presence of predators because of lower predation rate (while the ‘helmet phenotype’ is presumably costly in the absence of predator) (equation 2). Thus, producing the ‘helmet phenotype’ when predator chemical cues are detected in the environment should provide higher fitness than producing the ‘round head phenotype’, and reciprocally (equation 3). Hence, body shape plasticity in the presence of predators in *Daphnia lumholtzi* is expected to be selected for.



Figure 3: Morphological caste differences in the ant *Pheidologeton diversus*. Credits: antstore.net.

2. Adaptive plasticity

2.1 Advantages to being plastic

If phenotypic plasticity improves a genotype's fitness when environmental change occurs, phenotypic plasticity is considered to be adaptive (Pigliucci, 2001; DeWitt and Scheiner, 2004a; Ghalambor et al., 2007; Arnold et al., 2019). Adaptive plasticity implies that the reaction norm (see box 1) has been shaped by natural selection to produce the most adequate phenotype (i.e. closer to the phenotypic optimum) across a range of environmental conditions, requiring that the environmental cue predicts the future selective environment (i.e. be reliable). The five examples presented in section 1 (figures 2 – 5) are expected to be examples of adaptive plasticity. On the contrary, if the phenotypes produced across environmental conditions are not favoured by natural selection, the plastic response is considered to be non-adaptive. For example, low nutritive resource acquisition can reduce growth and/or reproduction, which is expected to be counter-selected. Lastly, if the expressed traits in the different environments are not related to fitness of individuals, plasticity is considered as neutral. Note however that distinguishing between adaptive, non-adaptive, and neutral plasticity is often challenging (Ghalambor et al., 2007). Proving that phenotypic plasticity is adaptive requires to demonstrate the positive link between plasticity and fitness. In other words, it requires to show that fixed (canalized) genotypes are on average less adapted over all encountered environments than plastic ones, and this involves the difficult task to compare fitness of the same genotypes in several environments (Pigliucci, 2001; DeWitt and Scheiner, 2004b; Ghalambor et al., 2007; Forsman, 2015; Arnold et al., 2019). In some cases, taking into account trade-offs between functions or across life-time allows understanding the benefit provided by plasticity. For example, in the case discussed above where low nutritive resource acquisition can reduce growth and/or reproduction, not reproducing when nutritive resources are scarce can allow maintaining adequate body condition for future reproductive attempts when the environmental conditions will be more favourable (i.e. survival – fecundity trade-off).

2.2 Costs and limits of phenotypic plasticity

Even when plastic responses to the environment produce fitter phenotypes, phenotypic plasticity may also be costly, because of for example information acquisition cost and/or development instability (Dewitt, 1998; Van Buskirk and Steiner, 2009; Murren et al., 2015). However, empirical studies seem to indicate that such costs are rare or weak (e.g. Dewitt, 1998; Scheiner and Berrigan, 1998; Johansson, 2002; Van Buskirk and Steiner, 2009, but see Auld et al., 2010). Phenotypic plasticity is also expected to be limited by low environmental cue reliability (when the environmental cue is a poor predictor of the selective environment), by the time-lag between the environmental cue and the production of the phenotype and/or by the limited developmental range

(Moran, 1992; Dewitt, 1998; Van Buskirk and Steiner, 2009; Auld et al., 2010; Murren et al., 2015). Because of costs and/or limits of plasticity, the evolved reaction norm slope is expected to have a lower absolute value than the reaction norm of the optimum phenotype against the environment (Dewitt, 1998; DeWitt and Scheiner, 2004b; Kleunen and Fischer, 2005; Valladares et al., 2007; Van Buskirk and Steiner, 2009; Auld et al., 2010; Murren et al., 2015). Lack of genetic variation in plasticity can also limit the evolution of a perfect plasticity (DeWitt and Scheiner, 2004b). Overall, as for other traits, the evolution of a given reaction norm will be the results of the trade-offs between the selective advantages, the costs and/or the limits of phenotypic plasticity.

3. Empirical challenges when investigating the evolution of plasticity

Describing, understanding and predicting the evolution of phenotypic plasticity requires: i) characterizing the environmental cue inducing phenotypic changes and the predictability of the environmental variation; ii) determining the relationship between plastic responses and fitness, so as to measure selection on plasticity; and finally, iii) quantifying the variability of phenotypic plasticity in a population and estimate whether it is heritable.

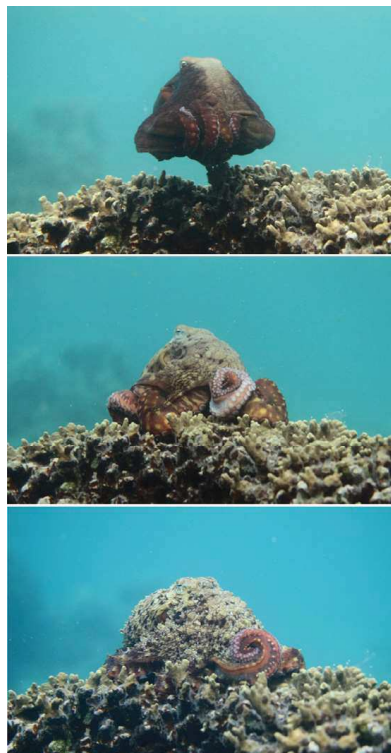


Figure 4: Change in colours, body shape and behaviour in the Octopus *Octopus cyanea* on the local environment. Dynamic camouflage in 2 seconds. Credits: Elisabeth Morcel (and see Bourjon Philippe, Sittler Alain-Pierre in: DORIS, 04/01/2017: Octopus cyanea Gray, 1849. <https://doris.ffesm.fr/ref/specie/2192>.

3.1 Cue reliability

An environmental cue for a plastic trait is an environmental factor that correlates with the future selective environment, and induces phenotypic changes. As selective environments and their environmental variability differ among ecosystems and populations, we expect that environmental cues and their reliability can change among natural populations, even within a species (Reed et al., 2010; Chevin and Lande, 2015). The environmental cues can differ by i) the environmental factor used to predict the future selection, e.g. temperature, humidity or population density; or ii) the time window when the environmental factor is taken into account to modulate the phenotype. For instance a population can be sensitive to temperature in spring or in summer to modulate their breeding phenology (e.g. Phillimore et al., 2016).

The most reliable cue is obviously the selective environment itself. However, production of the plastic response can take some time, such that the environmental cue needs to be detected before the selective environment, leading to a lag between detection of the cue and selection on the expressed phenotype. Theoretical predictions show that the reaction norm slope, which measures plasticity for a linear reaction norm, is expected to be proportional to the covariance between environmental cue and selective environment, such that plasticity decreases with lower reliable cues (Gavrilets and Scheiner, 1993; de Jong, 1999).



Figure 5: Two morphs of a single clone of *Daphia lumholtzi*. On the left, the morph developed in the presence of predators; on the right, the morph developed without cues for the presence of predators. Credits: (Agrawal, 2001).

Phenotypic plasticity is generally expected to be multidimensional, because using the multidimensional information provided by the habitats may allow better predictability of the future selective pressures (Chevin and Lande, 2015). Plasticity in response to several environmental gradients has been investigated in a number of case studies (Dorn et al., 2000; Griffith and Sultan,

2005; Kasumovic, 2013; Westneat et al., 2014; Bourret et al., 2015; Nilsson et al., 2019). For example, in tree swallow (*Tachycineta bicolor*), female lay later with increasing latitude, but earlier in warmer spring and in high breeder density. In addition, the spring temperature and the breeding density interact negatively, leading to a complex reaction norm of laying date (Bourret et al., 2015). Phenotypic plasticity may involve environmental cue(s) at several temporal scales. The phenotype can change according to environmental conditions at the beginning of the life (i.e. early-life effects) and/or be transgenerational, in response to the environmental cue(s) experienced by the parents. For such plasticity to evolve, the environmental fluctuations have to be predictable and with larger frequency than one generation (figure 6, Agrawal et al., 1999; Galloway and Etterson, 2007; Leimar and McNamara, 2015). However, phenotypic plasticity is usually studied with respect to only one environmental gradient, largely for methodological issues.

Box 1: The reaction norm framework

The way the phenotype changes with the environmental variation via plastic responses can be describe using two statistical frameworks: the character-state or the reaction norm frameworks. The character-state framework proposes to apprehend the phenotypic variation in response to the environment as discrete character states (e.g. Falconer, 1990) while the reaction norm framework (first proposed by Woltereck, 1909) conceptualizes the phenotypic variation with environment as a mathematical function of the trait in respect to a continuous environmental gradient, the environmental cue. The two approaches are mathematically related (Windig et al., 2004; Morrissey and Liefting, 2016) and the choice of one framework instead of the other is driven by the kind of plasticity and the phenotypic trait. Typically, the reaction norm framework is generally used for continuous phenotypic variation while the character state approach is used for categorical traits and threshold responses.

During this thesis we used the reaction norm framework (see e.g. Via and Lande, 1985; Nussey et al., 2007). Phenotypic plasticity of a given labile trait is often (satisfactorily) described as a linear reaction norm (e.g. Charmantier et al., 2008)

$$Z_{ij} = \mu + bE_j + e \quad (\text{b1})$$

where the trait Z of the individual i in the year j decrible as a function of its average phenotype μ and its plasticity b according to the environmental cue E of the year j , and e are residuals. Note however, that a reaction norm can take different shapes (David et al., 2004), such as sigmoid (e.g. Hammill et al., 2008). In the (linear) reaction norm framework, when the environmental cue is mean-centered (Morrissey

and Lieferting, 2016), the intercept of the regression of the trait on the cue is the average phenotype through the whole environmental gradient, while the slope estimates the phenotypic plasticity: the amount of trait change for one unit of environmental change (figure box 1). In the reaction norm framework, the phenotypic plasticity can be apprehended as a phenotypic trait *per se*. The (linear) reaction norm of a plastic trait and specifically its slope is then the focal trait in eco-evolutionary studies.

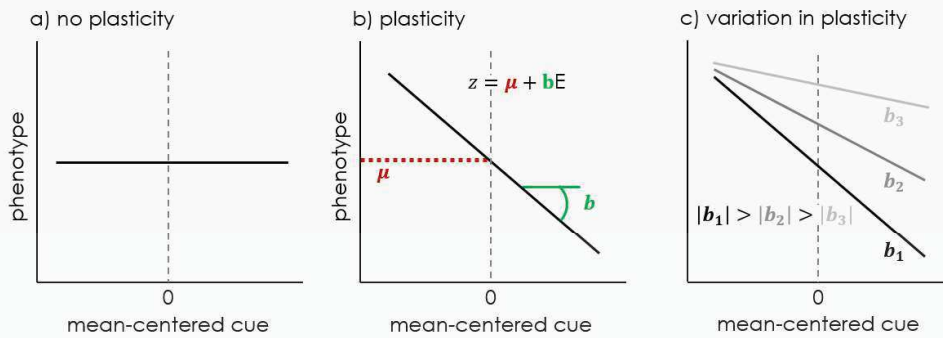


Figure Box 1: The reaction norm framework. a) No variation of the phenotype with the mean-centered environmental cue, that is no plasticity; b) (negative) Variation of the phenotype with the environmental cue describe as negative linear function with μ the elevation, corresponding to the average phenotype over the whole environmental variation (if the environmental cue is mean-centered) and b the slope, quantifying the phenotypic plasticity; c) Several strength of phenotypic plasticity: the more the slope absolute values b , stronger is the phenotypic change with the environmental cue.

Studying phenotypic plasticity requires to precisely characterize the cue that drives phenotypic variation. In natural populations, it is difficult (in some cases even impossible) to induce controlled environmental variation so as to identify which dimension of the environment is a cue for a specific plastic trait. Instead, cues are inferred from environmental parameter(s) (e.g. temperature and/or precipitation) that best correlate with phenotypic variation. ‘Sliding window analyses’ are generally used to characterize the time window during which the environmental parameter influences the phenotype. A user-friendly R package was recently implemented (Bailey and van de Pol, 2016; van de Pol et al., 2016), and see (Simmonds et al., 2019) for a recent comparison between several statistical approaches. Note however that such correlative approaches do not necessarily identify the cause of the phenotypic variation, but only a surrogate for it.

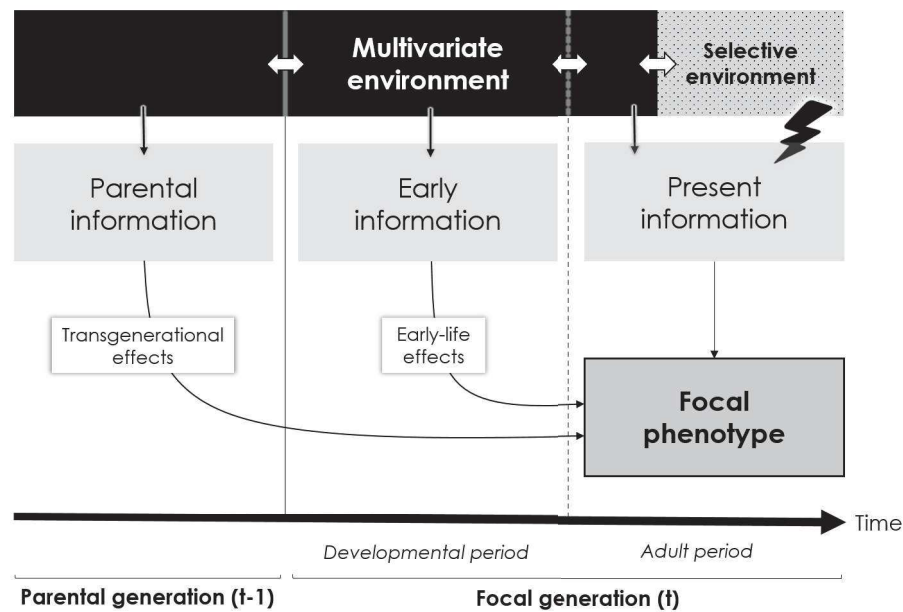


Figure 6: Summarized diagram of the relationships between the environment, cues, and phenotypes in the framework of phenotypic plasticity. The multivariate environment can be used as a cue to initiate a plastic response in an individual (thin black arrows). The focal phenotype (on which selection is acting in our example, black thunderbolt) can be affected by current environmental conditions, earlier life events and the preceding parental generation. Information about past environments (either parental or from early life) includes different sources, such as the phenotype itself (at the same or another trait), or epigenetics. For phenotypic plasticity to be adaptive, cues in the successive periods and generations have to be correlated with the future selective environment (white arrows). Modified from Bonamour et al., 2019.

3.2 Natural selection on phenotypic plasticity

Adaptive plasticity evolves when several phenotypes are favoured in several environmental conditions encountered by individuals in a population. If the selection on a trait changes with environmental conditions (e.g. under stabilising selection the optimal phenotype is a function of an environmental gradient) and if the plasticity is heritable, then natural selection will favour plastic genotypes indirectly through the environment-dependent selection on the traits expressed in the several environments (figure 7, Via and Lande, 1985; Lande, 2009). Adaptive plasticity is thus a by-product of the selection on the trait varying depending on the environment. The optimal reaction norm is the one that maximizes the fitness of individuals across all environmental conditions. If the plastic capacity presents heritable variance, that is among-genotype variation of the slope reaction norm, there will be evolution of plasticity in the population. If phenotypic plasticity is related to fitness and variable among-individuals, phenotypic plasticity is selected and selection gradient on plasticity can be estimated in wild populations (e.g. van Tien-deren and Koelewijn, 1994; Scheiner and Callahan, 1999; Scheiner et al., 2000;

Brommer et al., 2005, 2012; Nussey et al., 2005b; Hendry, 2016). Note that there are several proposed methods to estimate selection on plasticity yet no study so far has compared the efficiency of these different methods.

Because of the variable nature of ecosystems, and current global environmental changes, selection on plasticity is expected to be commonly observed in wild populations. We find only one proper meta-analysis of selection on plasticity estimating the average selection gradient on plasticity (Arnold et al., 2019). Contrary to expectations, results show no general pattern of selection for or against plasticity. However, we can find examples of significant selection for steeper reaction norm slope (more plasticity, e.g. Nicotra et al., 2015), shallower reaction norm slope (less plasticity, e.g. Stinchcombe et al., 2004), and even stabilizing selection on plasticity (e.g. Reed et al., 2006) among the few empirical studies. Note that this meta-analysis represents a small sample size of only eight studies and is restricted to plasticity in response to temperature. Also, even if plasticity is advantageous, low among-individual variation in plasticity and/or fitness will hamper the detection of selection for plasticity. In addition to these analyses, it is interesting to review other studies exploring the adaptiveness of plasticity via indirect methods. Notably, Davidson et al. (2011) test whether invasive species are more plastic than non-invasive ones, and Palacio-López et al. (2015) address the same question specifically on plant experiments using transplant designs: once again, results show no general pattern of selection on plasticity.

To be adaptive, phenotypic plasticity should have evolved under natural selection, requiring among-individual variation and heritability of the reaction norm. We should thus observe among -individual and -genotype variations of reaction norm in some wild populations.

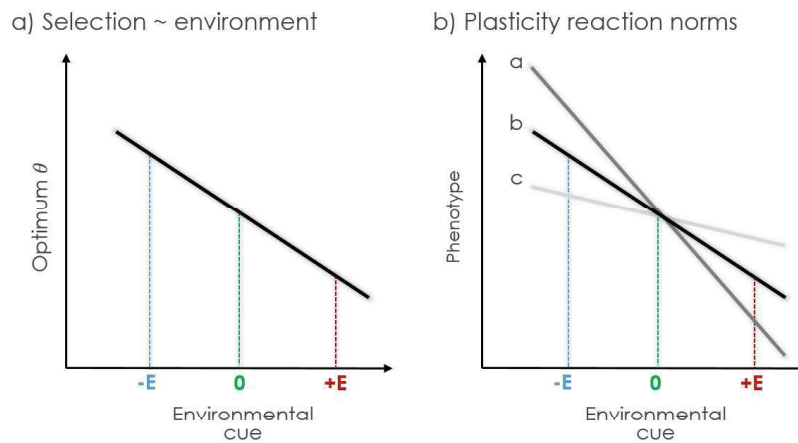


Figure 7: Most plastic genotypes induce more extreme phenotypes in extreme environments, driving selection on reaction norms. a) Selection varies depending on the environment, with different optimum phenotypes (θ) correlated to the reliable environmental cue. b) Three genotypes ‘a’, ‘b’ and ‘c’ with different reaction norm slopes (but same intercept, for the ease of the representation), with genotype ‘b’ the genotype displaying the optimal reaction norm (equal to the reaction norm of the optimum presented in panel (a) and thus allowing to express phenotypes in the different environments closest to the phenotypic optimum). The environmental cue varies from $-E$ (in blue) to $+E$ (in red) and is mean-centered, leading to the same expressed phenotype in the average 0 environment (in green), assuming no covariance between intercept and slope. In such a simple case, we can see that the most plastic genotype ‘a’ expresses more extreme phenotypes in both $-E$ and $+E$ environments compared to genotypes ‘b’ and ‘c’. Selection on plasticity results from the selection on the trait expressed in the different environments. In this case, genotype ‘a’ produces too extreme phenotypes compared to the optimum in the different environmental conditions and the reaction norm ‘a’ is under selection to be shallower. On the contrary, genotype ‘c’ produces too stable phenotypes and the reaction norm ‘c’ is thus selected to be steeper.

3.3 Variability of phenotypic plasticity

Among-individual variation

In natural populations there may be among-individual variation of plasticity, meaning that individuals differ in the way they modulate their trait expression according to environmental variation (i.e. among-individual variation in reaction norm slope, often symbolized as ‘ $I \times E$ ’, figure 8). Phenotypic variation in reaction norm parameters is a prerequisite for selection to operate on these traits. However, elevation and slope may also covary with each other (figure 9), thus constraining how selection acts on each of them. As an example, personality reaction norm studies often show that individuals with high aggressiveness do not (or little) adjust their behaviour according to environment, while the less aggressive individuals plastically modulate their phenotype, suggesting a negative covariance between intercept and slope of aggressiveness reaction norm (Koolhaas et al., 1999; Dingemanse et al., 2010).

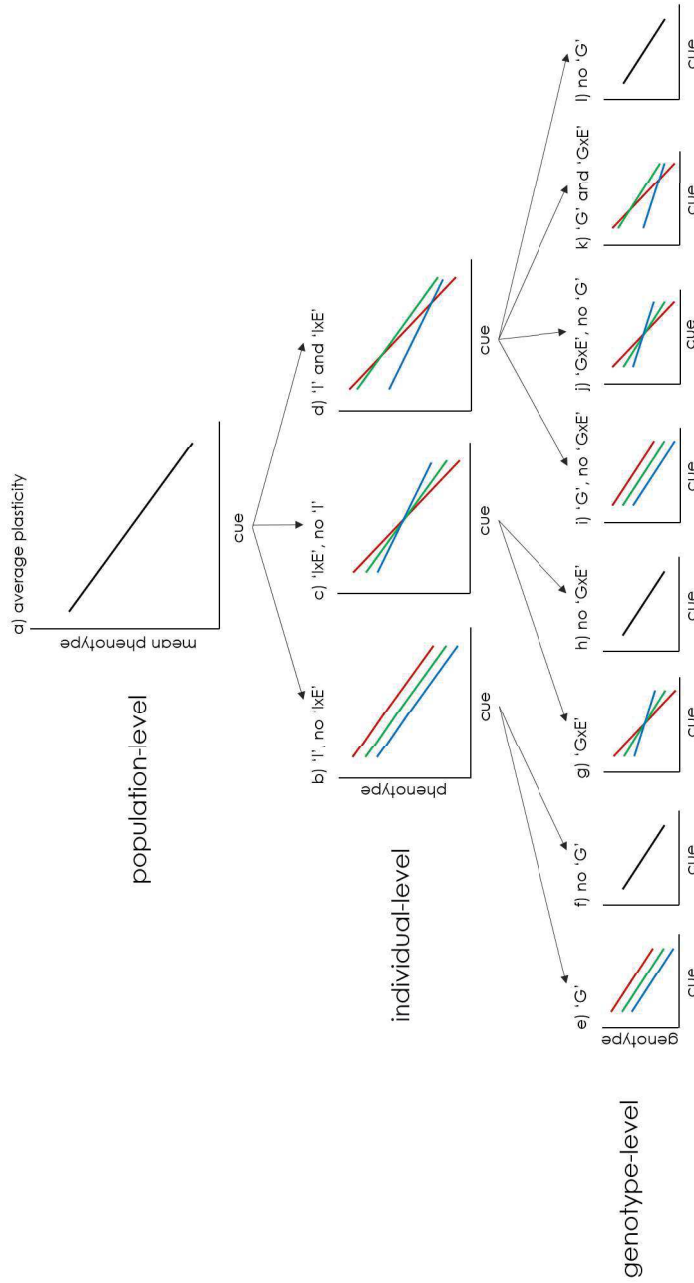


Figure 8: Variation in phenotypic plasticity among individuals and genotypes. a) Average phenotypic plasticity at the population level; b) Among-individual variation on average phenotype ('I'); c) Among-individual variation in plasticity ('IxE'); d) Among-individual variation of reaction norm, both elevation and slope ('I' and 'IxE'); among-individual variation in intercept, slope or both can be caused by among-genotype variation of e,i) intercept, g,j) slope or k) both; if there is no among-genotype variation f,h,l), the among-individual variation in intercept is caused by permanent environment (inspired by Nussey et al., 2007).

The (co)variances of reaction norm intercept and slope can be estimated using phenotypic random regression models (e.g. Gienapp and Brommer, 2014; Hayward et al., 2014; Abbey-Lee and Dingemanse, 2019; Brommer, 2013; Nussey et al., 2007; Martin et al., 2011), where the phenotype of an individual is explained by two individual random effects: one for the intercept and one for the slope of the regression with respect to an environmental factor (the cue). Among-individual variation of plasticity has been detected in many wild populations, especially in life-history traits (e.g. Brommer et al., 2005; Brommer and Rattiste, 2008; Nussey et al., 2005a; Husby et al., 2010; Porlier et al., 2012; Bourret et al., 2015). In addition, phenotypic covariance between elevation and slope of reaction norm have been observed in several wild populations (e.g. Brommer et al., 2005; Nussey et al., 2005b; Dingemanse et al., 2012).

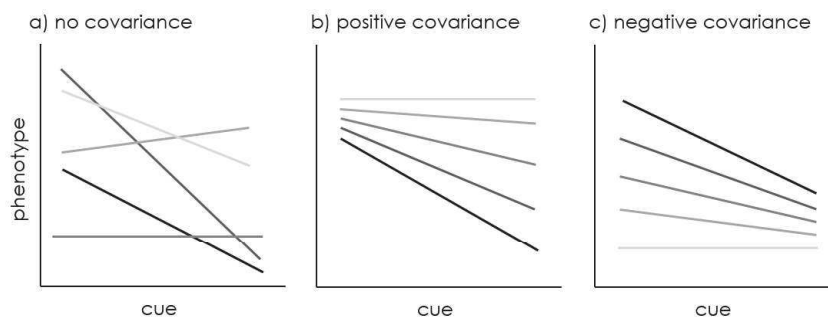


Figure 9: Covariance between intercept and slope of reaction norms. a) No covariance, b) Positive covariance, c) Negative covariance of negative linear reaction norms. In case of negative average reaction norm, a positive covariance between reaction norm intercept and slope means that individuals with high mean phenotype present on average higher value of reaction norm slope (i.e. lower plasticity) than individuals with low mean phenotype. On the contrary, a negative covariance between reaction norm intercept and slope means that individuals with high mean phenotype present on average lower slope (i.e. higher plasticity) than individuals with high mean phenotype.

Among-genotype variation

Differences in reaction norm slopes among-individuals may be heritable, and/or result from different environmental driver(s) (other than the identified environmental cue) or physiological condition experienced by individuals within the same population (Nussey et al., 2007). Evolution of reaction norms rests on among-individual variation with additive genetic origin, such that there among-genotype variation of intercept (symbolize as ‘G’) and/or slope (symbolized as ‘GxE’, e.g. Nussey et al., 2007, see figure 8, Lynch and Walsh, 1998).

Examples of heritable plasticity are common in laboratory (e.g. Barrett et al., 2011; Scott et al., 2018) but are more rarely detected in nature. Nevertheless, there has been increasing empirical evidence in the recent years, notably due to the emergence of long-term field monitoring with enough statistical power (e.g. Pelletier et al., 2007; Husby et al., 2010). As for individual-

level variation, the heritable part of the intercept and slope could covary (not necessarily in the same way than at the phenotypic level). The genetic covariation of the intercept and the slope reaction norm will affect the rate of its response to selection, just as for any genetic correlation between phenotypic traits. If reaction norm intercept and slope are selected in the same direction, then the response to selection will be facilitated if they are positively correlated. On the contrary, if selection is opposite between elevation and slope, response to selection will be constrained under positive correlation (and reciprocally with negative correlation). Using (usually social, or genetic since recently) pedigree, additive genetic (co)variance of average trait and plasticity can be estimated using random regression animal models (Kruuk, 2004 and see e.g., Nussey et al., 2005b; Husby et al., 2010; Brommer, 2013; Ramakers et al., 2018). Random regression animal models are random regressions where the phenotypic variance is partitioned into a permanent environment variance (i.e. individual random variance on the intercept and the slope) and an additive genetic effect (i.e. genetic random effect on the intercept and the slope), which are estimated using the genetic relationship between individuals.

There have been many debates about the genetic basis of plasticity (Windig et al., 2004). In particular, the scientific community today argues that the among-genotype variation (underlying the among-individual variation) in plasticity estimated by quantitative genetic models can in fact have both genetic and/or epigenetic bases, but these two sources of variation are rarely distinguished. 'Plasticity genes' (often regulatory genes, expressed differently depending on the environmental conditions, Schlichting and Pigliucci, 1993), plausibly acting at any cascade in the development of the phenotype, are generally expected to exist (Windig et al., 2004). In addition, empirical evidence of the role of epigenetic variation in the plasticity are accumulating (Ecker et al., 2018), notably in developmental plasticity of health and disease, especially in human (e.g. Feinberg, 2007; Laubach et al., 2018), in human neural plasticity (e.g. Ortuño-sahagún et al., 2019), or in fish developmental plasticity according to early life temperature (Jonsson and Jonsson, 2019). However, whatever the genetic and/or epigenetic origin of plasticity, if plasticity is a quantitative trait coded by a large number of genes (or epigenetic variants), quantitative genetic approaches such as random regression animal model should allow estimation of additive genetic variation of plasticity to predict its evolution. On the contrary, if plasticity is caused by a small number of genes (or epigenetic variants), we need to know what and how many genes underlying plasticity reaction norm shape to understand its evolution.

Within-individual variation

In the case of labile traits that can change repeatedly along life, in addition to among –individual and –genotype variation of plasticity, sensitivity to the environmental cue can vary during the lifetime of individuals. Such within-individual variation in phenotypic plasticity can be due to age-dependent variation of body condition and/or experience for example. For instance, the study of Grieco et al. (2002) suggests that responses to environmental conditions may

involve a learning process. They show that blue tits supplied with food during the reproductive period in a given year lay at the same period the next year, while blue tits without additional nutritive resource lay earlier in the next year. This study may show a learning process of laying date depending on the environmental conditions during the precedent breeding events. If phenotypic plasticity is (partly) learned, the most experimented individuals (i.e. the oldest ones) might display the best plasticity, closest to the optimal reaction norm. On the contrary, because plastic responses require resources in order to detect environmental variability and to produce the phenotype, individuals in poor body condition may display lower plasticity than individuals in high body condition. In particular, senescent individuals with overall decrease in performances may display less adaptive plasticity. In such a case, age-dependent plasticity could be considered as senescence of phenotypic plasticity. Within-individual variation of plasticity may have ultimately been moulded by natural selection, e.g. because being plastic at a given age may be more costly than at another period of life, or because of decreasing selection pressure in old ages, leading to senescence.

A few theoretical studies addressed age-dependent plasticity, and they generally predicted decreasing plasticity with age (e.g. Fischer et al., 2014; Fawcett and Frankenhuis, 2015; Stamps and Krishnan, 2017); note however that these studies mostly modeled binary environmental variations and/or traits, and focused on non-labile traits. We thus need formal theoretical predictions about age-dependent variation for labile traits under continuous environmental variation, such as laying date. Empirical evidences of within-individual trait variation are rare and mainly concern behavioural traits in the laboratory (e.g. Atwell and Wagner, 2014; Ericsson et al., 2016) with very scarce studies in natural populations (e.g. Araya-Ajoy and Dingemanse, 2016). The few studies that I found show decreasing plasticity with age (e.g. Atwell and Wagner, 2014; Araya-Ajoy and Dingemanse, 2016; Ericsson et al., 2016), as predicted by theoretical models.

4. Eco-evolutionary dynamics and phenotypic plasticity

Eco-evolutionary dynamic is a recently expanding research field based on the idea that ecology can influence evolution, and evolution can influence ecology, leading to complex interactions and possibly to feedback loops between both levels (e.g. Pelletier et al., 2018). Phenotypic plasticity, by mediating the influence of the environment (abiotic or biotic) on phenotypes that affect fitness, should be a pivotal phenomenon in eco-evolutionary dynamics (Hendry, 2016).

Phenotypic plasticity is crucial to population persistence and phenotypic rescue (Chevin et al., 2010, 2013; Ashander et al., 2016) because (when adaptive) plastic phenotypic variation decreases selection pressure on trait in variable environment when environmental cue remains reliable in the new encountered environmental conditions (Tufto, 2000; Michel et al., 2014), possibly

leading to 'plastic rescue' (Hendry, 2016). Adaptive plastic response should thus also facilitate colonization (Lande, 2015) and invasion processes (Davidson et al., 2011), reducing the fitness costs associated to live in colonized habitats with new environmental conditions, but empirical evidences do not always agree with this prediction (see Palacio-López and Gianoli, 2011). However, because phenotypic plasticity contributes to modifying natural selection on plastic traits, adaptive plasticity may slow down genetic response to selection (e.g. Falconer, 1981; Ghalambor et al., 2007, and see Oostra et al., 2018, for a recent empirical evidence in the context of climate change).

On the contrary, several hypotheses argued that phenotypic plasticity can facilitate adaptation. The main argument is that phenotypic plasticity, if it allow colonization of new habitat (but see Davidson et al., 2011), expose phenotypes to new selective pressures, and thus promotes evolutionary change. Also, when there is variation in reaction norm, plastic response can expose cryptic genetic variation, usually not expressed under normal environmental conditions, thus increasing genetic variance, potentially enhancing response to selection (e.g. Ashander et al., 2016).

Another potentially preponderant role of phenotypic plasticity in evolution is known as the 'plasticity first evolution' hypothesis (Levis and Pfennig, 2016). It proposes that new phenotypes are first produced via plastic response to new environments and, if adaptive, then becomes a fixed phenotype encoded in the genome and/or epigenome via genetic assimilation (Price et al., 2003; Ehrenreich and Pfennig, 2016; Levis and Pfennig, 2016; Pocheville and Étienne, 2017; Kelly, 2019). Hence, this hypothesis proposes that phenotypic innovations have plastic origins (West-Eberhard, 2003; Moczek et al., 2011). Levis et al. (2018) recently provided one of the rare empirical arguments of this hypothesis in the spadefoot toad tadpoles. The authors show that ancestral plasticity in the production of carnivorous morphs in response to diet was adaptively molded by natural selection to produce an evolutionary novelty, the production of carnivorous morph independently of the environmental conditions in the *Spea bombifrons* toad species.

Because of these potential roles of phenotypic plasticity in eco-evolutionary dynamics, plasticity is expected to matter in several ecological contexts. Especially, many empirical studies show that phenotypic plasticity has crucial role(s) in evolution at a broader scale (Hendry, 2016). Phenotypic plasticity it is involved in population differentiation (Schmid and Guillaume, 2017), as well as in diversification and speciation processes (Schlichting, 2004; Pfennig et al., 2010). Indeed, because phenotypic plasticity may allow colonisation of new habitats (Davidson et al., 2011), populations in several localities should be submitted to different selective regimes, leading to population differentiation and then possibly leads to speciation (West-Eberhard, 2003; Thibert-Plante and Hendry, 2011). However, the opposite can also be true: because of reduced selection pressure in the presence of adaptive plasticity, the rate of genetic response to selection can decrease, thus slowing down population differentiation (Price et al., 2003; Thibert-Plante and Hendry, 2011). However, several studies show that phenotypic plasticity may be higher in highly

diversified taxa. Note however that these studies cannot distinguish whether phenotypic plasticity promotes diversification, or conversely the diversification processes selects for higher phenotypic plasticity (Hendry, 2016).

Phenotypic plasticity also should play a role in species interactions (Agrawal, 2001), species coexistence (Turcotte and Levine, 2016) and community composition (Hendry, 2016) because plastic modulation allow changes in the realized ecological niche depending on (biotic and abiotic) environmental conditions. Closer to the topic of this thesis, plasticity is often involved in phenological (mis)match between trophic levels, e.g. predators and preys, (Visser and Gienapp, 2019) which could influence population dynamics (e. g. Reed et al., 2013b, but see Reed et al., 2013a).

5. Global change and phenotypic plasticity

Human activities cause environmental changes at the planetary scale. These changes are so tremendous that scientists define our era as the Anthropocene in the geologic scale (e.g. Lewis and Maslin, 2015). Living organisms are thus currently experiencing upheavals, such as urbanisation, pollution, and/or climate change. Virtually all such large-scale changes in the environment are expected to change selection pressures, driving evolution of natural populations (Vitousek et al., 1997; Alonso et al., 2001; Palumbi, 2001; Laporte et al., 2016; Pigeon et al., 2016; Fugère and Hendry, 2018). The new environmental conditions can be different from the environment where species have evolved in the long term, and the relationship between species may be modified, threatening the persistence of populations (e.g. Radchuk et al., 2019; Visser and Gienapp, 2019). Among the different components of human-induced global change, climate change has a particularly strong and general impact on natural populations all around the world (Walther et al., 2002; Parmesan and Yohe, 2003; Parmesan, 2006). Several meta-analyses show that organisms generally advance their phenology, especially for spring life-history events, because of global warming (e.g. Walther et al., 2002; Parmesan and Yohe, 2003; Radchuk et al., 2019). Phenotypic plasticity is one of the most important mechanism allowing population persistence in the context of climate change (Matesanz et al., 2010; Gienapp and Brommer, 2014; Merilä and Hendry, 2014; van Baaren and Candolin, 2018). This is especially true for phenological traits that condition fitness of individuals, and are often heritable and strongly related to climate, particularly in seasonal regions (Charmantier and Gienapp, 2014).

Despite allowing efficient response to environmental variation in the past, phenotypic plasticity may not be adaptive anymore in the new and/or extreme environmental conditions created by the rapid global change. Global change may change the reliability of environmental cues, by changing their correlation with the selective environment, decreasing the adaptive advantage to being plasticity, and selecting for lower plasticity (Gavrilets and Scheiner, 1993). When this occurs, the cue itself may evolve if it is heritable, leading to a change in the environmental parameter used (e.g. temperature, precipitation, or their combination), and/or the time window. In addition, organisms may

be submitted to new environmental conditions that had not been encountered during the evolutionary history of the species, such that plastic responses could produce non-adapted phenotype in the new environment (Ghalambor et al., 2007; Chevin and Hoffmann, 2017). Finally, even if phenotypic plasticity is still adaptive in the context of global change, the reaction norm may not allow producing the optimal phenotype because of too low plastic variation in response to the environment. In such a case, reaction norm will be selected for higher plasticity. A recent meta-analysis of (Radchuk et al., 2019) on wild vertebrates showed that adaptive responses to climate change (mediated by either microevolution and/or phenotypic plasticity) are overall insufficient to allow population persistence in the long run. In addition, plasticity may not be large enough to limit impact of global warming, at least in ectotherms (Gunderson and Stillman, 2015) and plants (Duputié et al., 2015).

Global changes are thus expected to often lead to selection on plasticity, with a direction that depends on the type of environmental change. Current scientific challenges are to assess whether phenotypic plasticity is sufficient to allow population persistence in a context of global change, and if not, to understand whether and how plasticity may evolve to help populations to cope with the new anthropogenic environmental variability.

6. Thesis outline

6.1 General objectives

Phenology, the annual timing of life-history events in a population, is crucial for population persistence, especially reproductive phenology. Phenology is generally plastic with climate and is one of the population characteristics most impacted by climate change (Parmesan and Yohe, 2003; Parmesan, 2006; Radchuk et al., 2019). Studying phenological plasticity in wild populations is thus important to understand impacts of climate change on population characteristics and persistence.

Despite representing only about 0.0004% of the total biomass on earth (c. 0.002 Giga tons of carbon, Bar-On et al., 2018), wild birds are present all around the world, in all ecosystems and were subject to a long history of ornithology, providing exceptional ecological and biological knowledge about of this taxon. The many long-term monitorings of wild bird populations allows showing that birds are highly impacted by climate change (Møller et al., 2019).

Laying date in birds, especially in passerines, is a textbook example of reproductive phenology in nature. Laying date is generally heritable (Postma, 2014) and plastic in response to spring environmental conditions, and among-year variation of laying date is particularly well correlated to early-spring temperature variation in temperate regions (e.g. Brommer and Rattiste, 2008; Phillimore et al., 2016). The timing of breeding is also generally closely related to fitness of individuals, and is often under negative directional selection for earlier breeding (e.g. Porlier et al., 2012), and can genetically evolve (e.g. Verhagen et al., 2019). We know that selection on laying date can be related to

climate conditions such as average and extreme events of temperature, which are increasingly common with climate change, as shown in a Mediterranean blue tit population (Marrot et al., 2017, 2018). In addition to climatic influence, annual reproductive success of birds is expected to be dependent on synchrony between their reproductive period and the period of development of the main nutritive resource for passerines chicks, the caterpillars (Blondel et al., 1991). The ‘birds – preys (mis)match’ is thus expected to be a driver of the selection for passerines in temperate regions (e.g. (Visser and Gienapp, 2019)). Therefore, the blue tit long-term study in Mediterranean region provides a great opportunity to study phenotypic plasticity of the reproductive phenology in a wild population.

During this thesis, I studied phenotypic plasticity of a repeated phenological trait, the laying date, in blue tits (*Cyanistes caeruleus*), a classic example of plasticity in the wild. I first studied the variability of environmental cue(s) and how climate change can influence their reliability (i.e. the capacity to predict the future selective environment), in a review of the literature and in a case study in the studied blue tit populations. Second, I explored the within-individual variability of laying date, according to environmental quality, age and spring temperature in search of ‘senescence of plasticity’ and ‘plasticity of senescence’. Third, I studied how selection and response to selection of the laying date and its plasticity can contribute to adaptation in an environment varying in time.

The study of phenotypic plasticity in controlled laboratory experiment is undoubtedly essential and probably methodologically easier than in wild populations. Nevertheless, we need to embrace the whole environmental effects on the phenotypic variation occurring in natural populations to understand ecological processes and predict evolutionary trajectories, and this can only be done in relevant natural conditions where wild populations are living. The same holds for the evolutionary potential assessed through estimation of additive genetic variance, natural selection and response to selection. As typical in studies of quantitative genetics in wild populations (Clutton-Brock and Sheldon, 2010), the study of phenotypic plasticity and its evolutionary potential require very large data set with several phenotypic observations on the same individuals (Martin and Festa-Bianchet, 2011; van de Pol, 2012), typically resulting from long-term longitudinal field-monitoring.

6.2 Long-term monitoring of (Mediterranean) blue tits

Study sites. The blue tit study in the French Mediterranean region provides a rare opportunity to explore phenotypic plasticity in a natural environment. Blue tits (*Cyanistes caeruleus*) are common passerines in the west Palearctic, inhabiting a large range of ecosystems such as forests, agricultural areas, gardens and parks in the countryside as well as cities. A long-term monitoring of blue tit populations in the South of France was initiated by Jacques Blondel in 1976. We focus in this thesis on four blue tit populations in this region (figure 10): the D-Rouvière (close to Montpellier in mainland France, monitored since 1991) and the D-Muro (in Corsica island, monitored since 1993) populations

inhabit forests dominated by Deciduous downy oaks (*Quercus pubescens*, hence the ‘D’), while the E-Pirio and the E-Muro populations (both in Corsica island, monitored since 1976 and 1998 respectively) inhabit forests dominated by Evergreen holm oaks (*Quercus ilex*, hence the ‘E’). The two types of forest ecosystems influence life-history traits in blue tits and birds are locally adapted to each habitat (Blondel et al., 2006; Charmantier et al., 2016).

Blue tit breeding season. Every spring, females and a males build a nest (mainly with moss in forest), and when the nest is finished females lay the clutch. Clutch size ranges generally from 5 to 15 eggs depending on the environmental conditions and the population. After the entire clutch is laid, the females incubate the eggs during 14 days. From the chicks hatching until they fledge outside of the nest and take their independence, parents feed them mainly with caterpillars, but also other preys such as spiders (Blondel et al., 1991). See figure 11 for schematic representations of the blue tit reproductive season. Estimations show that one chick need to be fed by about 1800 caterpillars during its development (Blondel et al., 1991; Tremblay et al., 2005). The maximal nutritive food demand of young birds is around 9 days (figure 11). The synchronisation between the caterpillar peak date of abundance and the chick development is thus expected to be crucial for chick body condition and reproductive success of the parent (e.g. Visser et al., 2006).

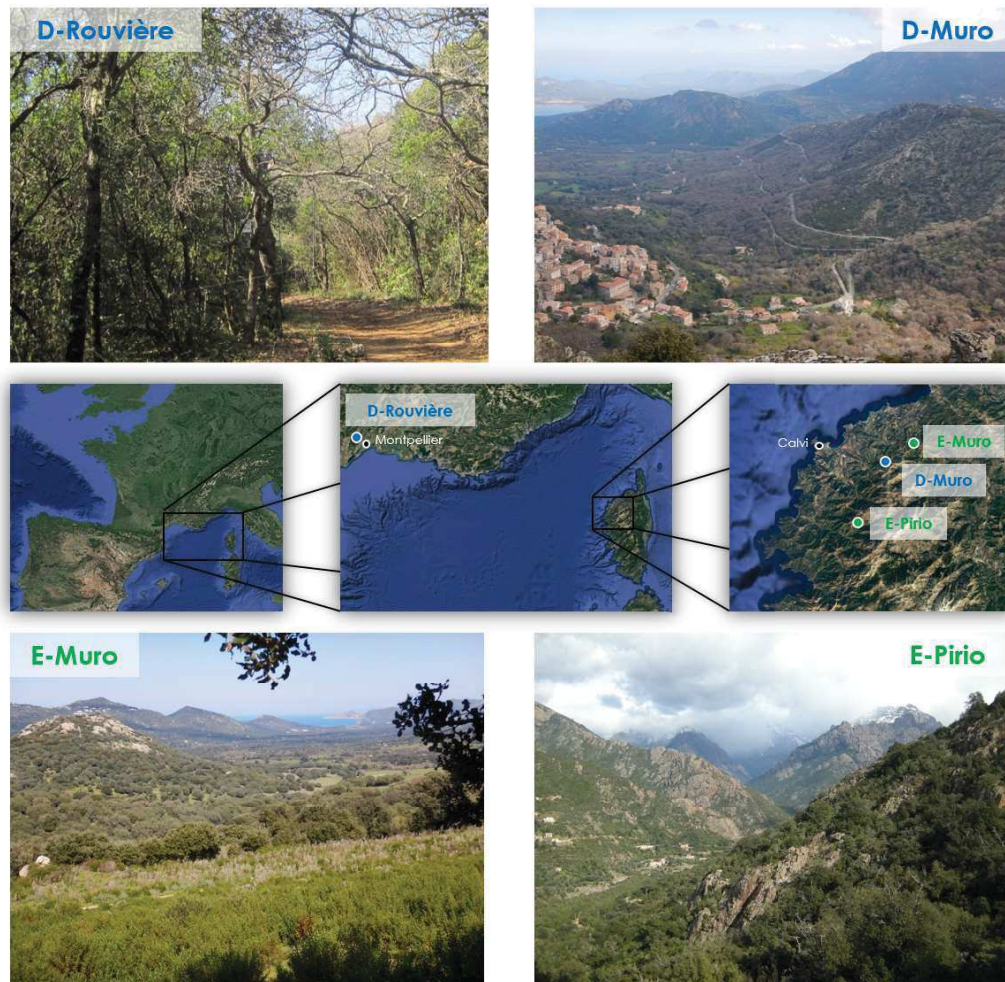


Figure 10: The Blue tit study sites. In the mainland France, the D-Rouvière population. In Corsica, the D-Muro, the E-Muro and the E-Pirio populations. The D-Rouvière and the D-Muro populations are located in areas mainly populated by deciduous oaks (hence the ‘D’), while the E-Pirio and the E-Muro population is in an area mainly dominated by evergreen oaks (hence the ‘E’). (Credits: Anne Charmantier and Christophe de Franceschi).

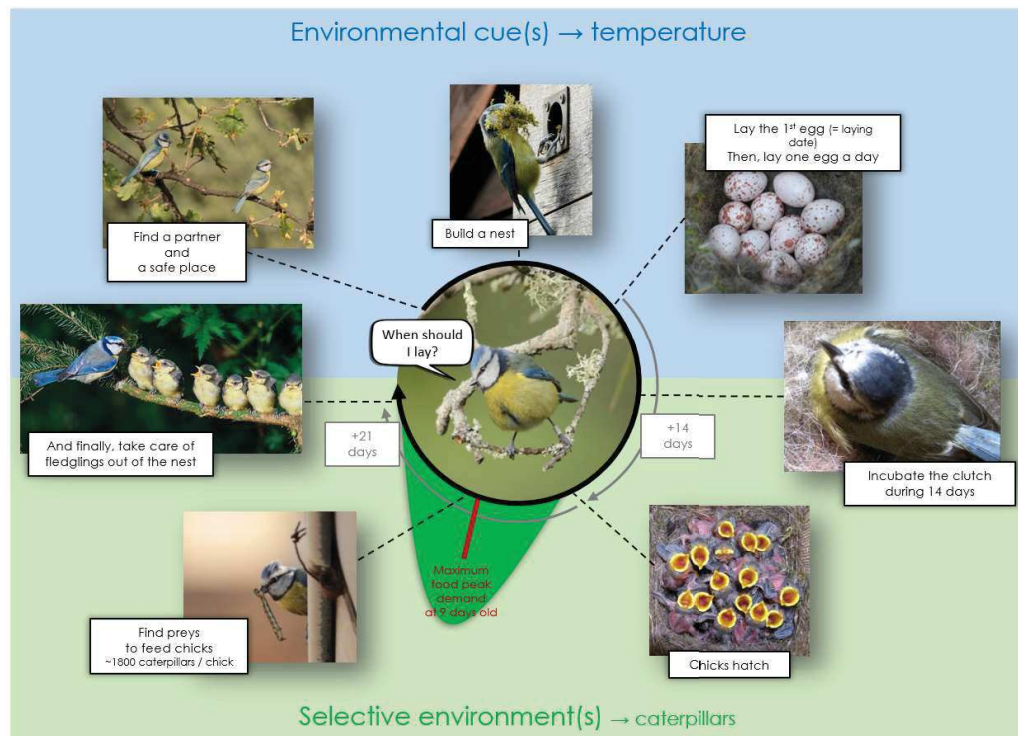


Figure 11: The blue tit breeding season. The reproductive period begins by the formation of a couple. Then, the male and female build their nest in a cavity. When the nest is ready, the female lays one egg a day and incubates the completed clutch for 14 days before the chicks hatch. During chick growth, both parents bring preys to feed their offspring. The main prey of the chicks is caterpillar and the chick's highest nutritive demands are around 9 days old. The chicks fledge 21 days after hatching. During several days, parents often take care of their chicks outside of the nest. The timing of the reproductive period and especially the date at which the first egg is laid is influenced by environmental cues, such as temperature. The reproductive success of the parents is dependent on the selective environments, determined to a large extent by the synchronisation between their timing of breeding and the timing of caterpillar development. The most studied selective environmental driver in blue tits (as in other passerines) is the 'bird – prey (mis)match', that is the time between the caterpillar peak date of abundance and the date when chicks are around 9 days old. (Credits: Anne Charmantier and Christophe de Franceschi).

Data collection. During the breeding season, each bird pair laying in the nest-boxes provided in forests (figure 12a) are monitored. Note that birds are free-living but the nest-boxes are human-made concrete, which may affect birds phenotypes, although this potential bias cannot be estimated in our experimental design (Møller, 1992). Also, population density increases with the number of nest-boxes provided in the forest, which should have impacts on population dynamics. However, we do not know what was the natural density of blue tits in our forest before the monitoring. Overall, these blue tits populations can be qualified of semi-wild populations. Every week, all nest-boxes are checked to collect the laying date, the number of eggs laid and the number of fledglings leaving the nest for each breeding attempt. In addition to phenological and reproductive success data, we collected usual morphological measurements in birds such as tarsus length and wing length, and some blood samples (figure 12b). Chicks are ringed with unique numbers at the nest, as are the parents when they are feeding them (figure 12c). The monitoring also allows producing a social pedigree for quantitative genetic analyses. Previous studies of Charmantier and collaborators show high extra-pair paternity rate in our populations (18-25% in Corsica, Charmantier et al., 2004, which is positively correlated to population density, Charmantier and Perret, 2004). The heritabilities are thus underestimated in our studied populations since they are based on social rather genetic pedigrees, but this underestimation is expected to be reasonable (less than 15% (Charmantier and Réale, 2005; Firth et al., 2015), but see (Bourret and Garant, 2017)). In addition to blue tits monitoring, we collected data about caterpillar phenology. Caterpillar frass mass is collected using coprometers (square of 50cm x 50cm with a frass collector, figure 12d) during the bird's breeding season allowing to estimate caterpillar abundance. The caterpillar peak date of abundance is the day with the highest quantity of collected frass.

Natural selection. The main environmental cue for laying date is early-spring temperature for blue tits (e.g. Porlier et al., 2012, as for passerine in temperature region in general (Visser et al., 2009; Caro et al., 2013b,a)). Laying date is under negative directional selection in all the four studied populations (Porlier et al., 2012; Marrot et al., 2017, 2018), meaning that earlier individuals have higher annual reproductive success than later ones (as generally found in other passerines and bird populations). Laying date is expected to be under stabilising selection (as in great tit e.g. Chevin et al., 2015), and negative quadratic selection has been detected in the E-Pirio population (de Villemereuil, in prep.), suggesting the existence of an optimal laying date providing highest annual reproductive success. Because of the link between bird reproductive phenology and the peak date of caterpillar abundance (which is also plastic with respect to environmental variation, e.g. van Asch and Visser, 2007), the 'bird - caterpillar (mis)match' is expected to be a key driver of selection on laying date, i.e. it is one of the dimensions of the 'selective environment' (Reed et al., 2013a,b; Ramakers et al., 2018; Visser and Gienapp, 2019).



Figure 12: The blue tit field work. a) Nest-boxes are checked every week to collect breeding information such as phenology (laying date and hatch date) or breeding success (number of hatchlings, fledglings); b) the field work material to ring and measure individuals and to perform blood sampling; c) Ringing of a male blue tit; d) Coprometers to collect caterpillar frass. (Credits: Anne Charmantier and Johanna Theys)

6.3 Roadmap

During this thesis, I asked how and why phenotypic plasticity of reproductive phenology occurs in blue tits. I studied variability, heritability and selection of laying date phenotypic plasticity in wild blue tit populations. While **chapter I** is about comparison of the four studied Mediterranean blue tit populations, the next chapters only focus on the longest studied population, from E-Pirio station. In **chapter One** (*manuscript A*, see figure 13 for a schematic summary of the thesis), I first review the impact of global change on environmental cue variation and reliability (i.e. the capacity to predict the future selective environment) in wild populations. I then detail a case study on the variability of environmental cue(s) among four blue tit and two caterpillar populations, and estimate reliability of blue tit cues in the context of climate change. In **chapter Two**, I investigate within-individual variation in laying date plasticity over lifetime, in two manuscripts. In *manuscript B*, I explore the age-dependence of laying date plasticity and annual reproductive success, in search for evidence of senescence of plasticity. In *manuscript C*, I ask whether the environmental quality experienced by females in their past affects their senes-

cence trajectories, using the concept of ‘environmental age’. This could be described as plasticity of senescence. **Chapter three** deals with selection on laying date and its plasticity, with two manuscripts. In *manuscript D*, I propose theoretical and methodological developments about measuring selection and response to selection on characters with skewed distributions, such as laying date, and discuss implications for understanding the paradox of stasis. In the last *manuscript E*, I investigate how natural selection and genetic response to selection of reaction norm elevation and slope (plasticity) may contribute to track patterns in fluctuating environment. Finally, I discuss results of this thesis in a general discussion and propose future directions for research.

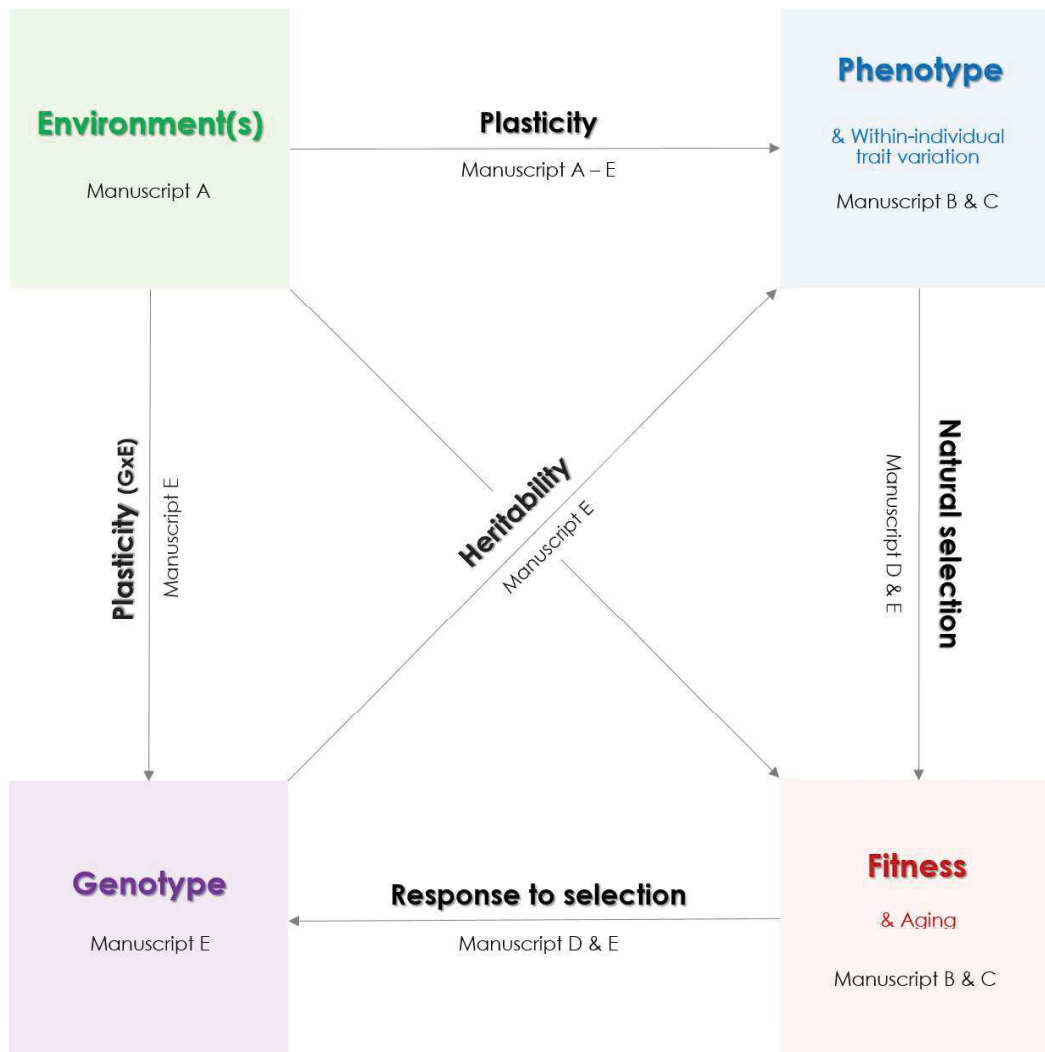


Figure 13: Thesis roadmap. The multivariate environment influences evolution in nature, via effects on phenotypes, fitness and genotypes. A given phenotype is the results of the genotype and inter-individual variation in phenotypes due to variation in genotypes is related to the heritability of the trait. Phenotypic variation due to environmental variation corresponds to phenotypic plasticity (with sometimes variation in plasticity among-individuals ‘I×E’ and genotypes G×E). A given phenotype can influence fitness via survival and/or fecundity. The environment can be a selective driver at the origin of natural selection, influencing fitness of individuals depending on their phenotypes. Natural selection results in a response to selection in the mean or variance of a trait if this trait is heritable. The environment has thus indirect influence on the genotypic variation in the population. Phenotypes and fitness can vary during the lifetime of individuals, inducing within-individual variation of trait (and/or within-individual variation of plasticity), and aging (or senescence) respectively.

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The Fango delta (Corsica).

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Chapter One

Cue reliability and global change

MANUSCRIPT A

Phenotypic plasticity in response to climate change: the importance of cue variation

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Abstract

Phenotypic plasticity is a major mechanism of response to global change. However, current plastic responses will only remain adaptive under future conditions if informative environmental cues are still available. We briefly summarize current knowledge of the evolutionary origin and mechanistic underpinnings of environmental cues for phenotypic plasticity, before highlighting the potentially complex effects of global change on cue availability and reliability. We then illustrate some of these aspects with a case study, comparing plasticity of avian phenology (blue tits) in two contrasted habitats: evergreen and deciduous forests. Using long-term data sets, we investigate the climatic factors linked to the breeding phenology of the birds and their main food source. Blue tits occupying different habitats differ extensively in the cues affecting laying date plasticity, as well as in the reliability of these cues as predictors of the putative driver of selective pressure, the date of caterpillar peak. The temporal trend for earlier laying date, detected only in the evergreen populations, is explained by increased temperature during their cue windows. Our results highlight the importance of integrating ecological mechanisms shaping variation in plasticity if we are to understand how global change will affect plasticity and its consequences on population biology.

Key words: Phenotypic plasticity, Climate change, Cue reliability, Blue tit, Laying date

1. Introduction

Global change encompasses modifications of the environment both at a global scale (e.g. climate) and at a local scale but in so large proportion that the whole planet is affected (e.g. urbanization, invasive species). It thus represents complex modifications of the environments in which populations face radically new conditions (e.g. pesticides, roads), but also new modalities of historically known environments (e.g. extreme climatic events, invasive predator). Studying whether and how wild organisms adapt to these rapid environmental changes is both an opportunity for in-depth evolutionary ecology scrutiny, and a societal challenge.

Phenotypic plasticity is a major mechanism of response to environmental variability, which may allow organisms to cope with rapid environmental changes, including global change. It has indeed been identified as the main mechanism of phenotypic change in response to climate change (Gienapp et al., 2007; Matesanz et al., 2010; Merilä and Hendry, 2014) and other human-induced rapid changes such as urbanization (Alberti et al., 2017). Phenotypic plasticity is also suspected to have an important role in colonization of new environments, geographic range shifts, and the success of invasive species (Davidson et al., 2011; Lande, 2015).

However, whether or not plasticity will aid adaptation and population persistence in a new environment depends on whether it is adaptive there. When it is adaptive, phenotypic plasticity can increase the probability of population persistence (Chevin et al., 2010), as exemplified in two great tit (*Parus major*) populations where phenotypic plasticity is predicted to increase the likelihood of population persistence under various scenarios of climate change (Vedder et al., 2013; Gienapp et al., 2013). However, these predictions are based on models that generally consider a “static” estimate of plasticity, without taking into account potential changes in reaction norms due to plasticity evolution, or the possibility that the adaptive nature of plasticity may be altered under new environmental conditions (Ghalambor et al., 2007), as is the case under global change.

A core prerequisite for adaptive predictive plasticity is the existence of reliable cues, i.e. accurate environmental information about future selection on the expressed plastic phenotype (Moran, 1992; Tufto, 2000; Botero et al., 2015; Scheiner, 2013). While the definition of cues is fairly straightforward in theory, their empirical characterization may be a very arduous task (electronic supplementary material, Box S1). Yet, it is a crucial step in order to understand how global change can affect adaptive plasticity and its consequences on population biology. For instance, theory predicts that if cue reliability decreases, plasticity may be selected to decrease (Gavrilets and Scheiner, 1993), or else may drive populations to extinction by increasing the expected load caused by phenotypic mismatch with the optimum in a fluctuating environment (Reed et al., 2010; Chevin et al., 2013; Ashander et al., 2016). This is just one possible scenario, but it illustrates the point that one of the most central tasks for understanding and predicting the ecological and evolutionary roles of pheno-

typic plasticity under global change is to decipher which environmental cues are used by organisms, why (i.e. which selective pressures), and the extent to which global change is likely to affect cue reliability.

To address these questions, we will start with a brief overview of the nature and evolution of cues in wild populations. We will then outline the potentially complex effects of global change on plasticity, through its effect on the availability and reliability of cues. Finally, we will illustrate some of these aspects with a case study comparing plasticity of phenology in four blue tits populations from contrasted habitats.

2. Nature and evolution of cues for phenotypic plasticity

For plasticity to be adaptive, the environment influencing the development and/or expression of a particular phenotypic trait (or set thereof) needs to be a reliable cue for the selective pressure on this trait. In a variable environment, this reliability implies that the cue(s) should predict the selective environment, i.e. the environmental conditions driving natural selection on the expressed plastic trait (figure 1 and Moran, 1992; Tufto, 2000; Scheiner, 2013), so that the plastic response leads to increased fitness (Ghalambor et al., 2007). Reliable cues do not require that the same environmental variable affects both the expression of a trait and selection on this trait: any environmental variable that is correlated with the environment of selection through space or time can act as a reliable cue for phenotypic plasticity (e.g. red/infra-red ratio signaling competitor presence, Griffith and Sultan, 2005). In fact, environmental variables that are most informative about the selective environment at the time of phenotypic determination may differ from the selective environment itself at this time (Chevin and Lande, 2015). For instance, rain may be a better predictor of the peak of food abundance at time $t + \tau$ (where τ is the lag between phenotypic determination and selection on the trait) than is food abundance itself at time t . More generally, organisms are expected to respond to the linear combination of environmental variables that best predicts variation in the phenotypic optimum (Gavrilets and Scheiner, 1993; Chevin and Lande, 2015). Very few case studies have investigated how an optimum phenotype is predicted by one environmental variable (Chevin et al., 2015), and probably even fewer address the joint effect of several environmental variables. In contrast, many empirical studies have focused on the multidimensional aspect of plasticity, and highlighted that organisms respond to different environmental factors in an integrated way (Westneat et al., 2009; Sgrò et al., 2016; Valladares et al., 2007; Polgar and Primack, 2011). Responding to multiple cues allows organisms to fine tune their phenotype in complex environments: for example, preys can be simultaneously confronted to different types of predators (Black, 1993; Teplitsky et al., 2004), or plants face the need to respond to herbivory as well as to competition for light and water availability (Valladares et al., 2007).

The aspect of cue reliability that influences the evolution of plasticity (in

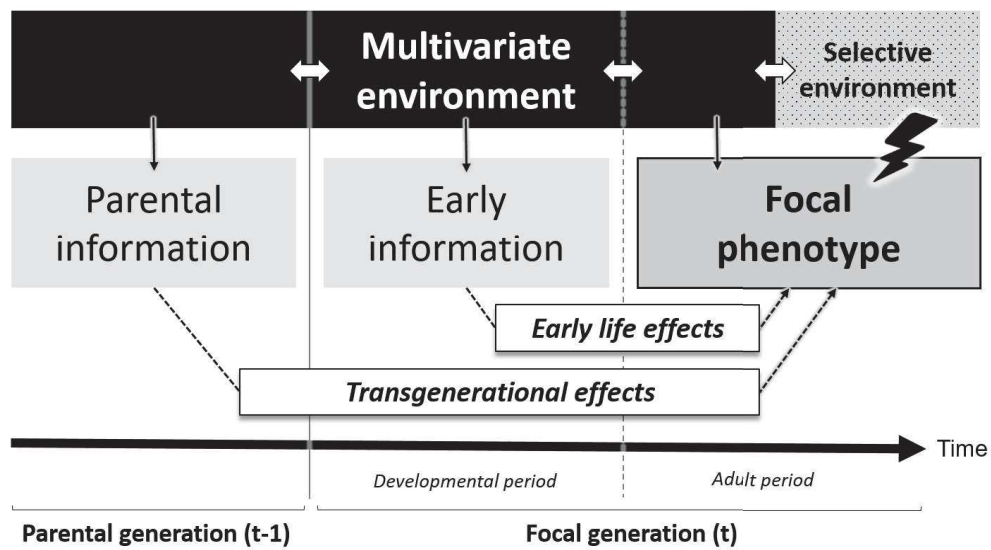


Figure 1: Summarized diagram of the relationships between the environment, cues, and phenotypes in the framework of phenotypic plasticity. The multivariate environment can be used as a cue to initiate a plastic response in an individual (thin black arrows). The focal phenotype (on which selection is acting in our example, black thunderbolt) can be affected by current environmental conditions, earlier life events and the preceding parental generation. Information about past environments (either parental or from early life) includes different sources, such as the phenotype itself (at the same or another trait), or epigenetics. For phenotypic plasticity to be adaptive, cues in the successive periods and generations have to be correlated with the future selective environment (white arrows).

models of linear reaction norms) is the regression slope of the environment of selection on the environment of development (Gavrilets and Scheiner, 1993; de Jong, 1999). When the time lag between these two environments increases, cues are expected to become less informative (Bradshaw, 1965; Padilla and Adolph, 1996), to an extent that depends on the time scale of environmental fluctuations: strong temporal autocorrelation of the environment may allow the evolution of plastic responses to early cues. Similarly, dispersal between cue perception and selection causes a spatial lag analog to the time lag (Moran, 1992), with an effect on plasticity evolution that depends on the degree of spatial autocorrelation over the typical dispersal distance. However, even a cue that reliably predicts the environment of selection can lead to maladaptive plastic responses, if there is a development lag (time between cue perception and phenotype expression, Scheiner, 2013), such that the “set value” corresponding to that cue is not reached when selection operates on the trait. When the environment fluctuates on time scales longer than the generation time and dispersal is low, information about parental environment can be a reliable cue for offspring, and transgenerational plasticity is expected to evolve (figure 1, Mousseau and Fox, 1998; Leimar and McNamara, 2015; McNamara et al., 2016). Transgenerational effects may be especially important for traits that are expressed and fixed early in the ontogeny (including many morphological traits), because offspring may lack sensory abilities during development (Uller, 2008), and have fewer time to integrate information about the environment. For example, in the nematode *Caenorhabditis elegans*, maternal provisioning in glycogen increases offspring fitness under anoxia. Using experimental evolution, Dey et al. (2016) showed that when normoxia (i.e. normal levels of oxygen) and anoxia (< 1% oxygen) were predictably alternating, mothers experiencing normoxia increased glycogen provisioning to their embryos (and vice versa for anoxia), demonstrating the evolution of maternal effects under predictable variation of the environment. In marine sticklebacks (*Gasterosteus aculeatus*) where body size is a key component of fitness, maternal effects could help adaptation to increased temperature under climate change, as offspring from mothers acclimated to 21°C were larger in this warm environment than conspecifics from mothers acclimated to 17°C, thanks to different mitochondrial performances (Shama et al., 2014).

Recently, there has been a growing interest in understanding theoretically how cue variability and reliability at different time scales interact to shape plastic responses. Organisms are predicted to integrate various sources of information, including cues from the current environment (for labile traits that change continuously in life), earlier cues perceived during development (early-life or carry-over effects), and transgenerational cues from the environment to which previous generations were exposed (figure 1, McNamara et al., 2016), including the parental phenotype itself (Kuijper and Hoyle, 2015). The relative weight of these different types of cues is expected to vary depending on their predictive power over different time scales (related to the temporal autocorrelation of the environment), and the strength of selection (Leimar and McNamara, 2015; McNamara et al., 2016; Kuijper and Hoyle, 2015).

Cue variation through space and/or time can lead to variation in plasticity, with an alternation of different ecotypes. A telling example of within-population variation in plasticity comes from the wild mosquitofish (*Gambusia holbrooki*). Fish born in the early spring (development at cool temperature) are expected to experience more variable environments than fish born in early summer (development at warm temperature). Accordingly, metabolic rate is plastic in fish born in early spring, but not in early summer fish (Seebacher et al., 2014). Following on from such encouraging case studies, more work is needed to assess, both theoretically and empirically, whether and how much variation in cue variability and predictability drives within-species variation in plasticity.

3. How may global change influence cues and plasticity?

Under global change, several aspects of the environment are expected to be modified, notably through the emergence of new environments (due to local habitat change or dispersal in new habitats), alteration of covariances among environmental variables, and increased climatic variability. These environmental changes can be gradual, but abrupt changes are also increasingly likely because of anthropogenic pressures (Ratajczak et al., 2018), e.g. in the form of extreme climate events such as floods, droughts, or storms. Altogether, environmental changes may affect the expression and evolution of plasticity through changes in cue reliability, cue perception and interpretation, and development of the phenotype.

3.1 Changes in the availability and reliability of cues

The simplest way environmental change may affect cue use is by disrupting their detection, either by degrading the signal or by disturbing organisms' sensory abilities. For example, juvenile damselfish (*Pomacentrus wardi*) no longer respond to predator cues in degraded environments (dead coral reefs), and the likely mechanism is a degradation of the conspecific alarm cue (Lönnstedt et al., 2013). Similarly, eutrophication can impair visual signals, leading to maladaptive plasticity in male sticklebacks (*Gasterosteus aculeatus*), as their increased investment in courtship behavior is not correlated with female interest (Candolin, 2009). Pervasive electromog, i.e. human-made electromagnetic noise, completely disrupts the magnetic compass orientation in the migratory European robin *Erithacus rubecula* (Engels et al., 2014).

When cues are still available, their reliability can be affected if the new environment is similar in some ways to a known environment. In the most extreme cases, a new environment is generating a signal similar to a previously known cue, but completely uncorrelated with both the original cue and the environment of selection. For example asphalt and ponds polarize light in the same way, leading mayflies to lay their eggs on the road rather than ponds

(Kriska et al., 1998), and insecticides can trigger costly morphological defenses in *Daphnia ambigua* in the absence of predators (Hanazato, 1991; Riessen, 2012). Such maladaptive responses based on cues that were previously reliable are termed evolutionary traps (Robertson et al., 2013; Schlaepfer et al., 2002). These traps will be all the more effective as the previous cue was highly reliable (Robertson et al., 2013).

On the other end, the cue can be sampled from the same historical environmental variable, but the correlation between this variable and the selective environment may have decreased or entirely vanished. This is expected under climate change, for instance because of increased climate stochasticity within years, or because the average temperature is not changing at the same rate for all seasons (Marrot et al., 2018; Williams et al., 2015). For example, the yellow bellied marmot (*Marmota flaviventris*) has gradually emerged earlier from hibernation (study led between 1975 and 1999) because of warmer air temperature earlier in the spring. However, the date of snowmelt has not changed in the Rocky Mountains, leading to lower foraging opportunities at emergence. While warmer air was historically a good predictor of snow melt, climate change has led to a mismatch between air temperature and snow cover (Inouye et al., 2000).

Because species are embedded in networks of ecological interactions, loss of cue reliability may arise from species responding differently to environmental changes, or interacting species responding to different cues. Changes in phenology are the most ubiquitous responses to climate change (Parmesan, 2006), and the best documented cases of disruption of ecological networks are based on a loss of synchrony between ecological interactors, such as predators and their preys, or plants and their pollinators (Visser and Both, 2005; Byers, 2017). If interacting species are responding to the same cue, their phenological mismatch may increase because their reaction norms are different. This is generally the case in trophic interactions, where consumers tend to display shallower (flatter) reaction norms to climate than their resources (Thackeray et al., 2016). This occurs because consumers generally have longer generation times than resources (due to larger body size), and thus longer developmental lags for the expression of the plastic trait, which reduces their ability to predict the environment of selection, as temporal autocorrelation affecting cue reliability decreases on average with longer time lags (Gienapp et al., 2014). Another possibility is that interacting species are actually using different cues that undergo different time trends. For example, four tree species of *Prunus* in Japan are flowering earlier due to increased temperatures during the time window most related to their phenology, yet temperature remains unchanged during the time window related to the phenology of the butterfly *Pieris rapae* that uses them as host plants. This leads to strong plasticity of phenology in trees but not in butterflies (Doi et al., 2008), resulting in an increasing plant-insect phenology mismatch due to warming (see also our case study below).

Some mechanisms may mitigate the loss of reliability in some cues. First, the potentially negative effects of loss of reliability of a particular environmental variable as a cue could be greatly alleviated if organisms are using multiple

cues, because partial redundancy in information from different environmental sources may increase the robustness of the signal (Dore et al., 2018). For example in the above-mentioned case of the coral reef damselfish, the loss of olfactory cue in dead coral water can be compensated by the use of visual cues (Lönstedt et al., 2013). Similarly, we could expect that the mismatch in trophic webs could be alleviated if species are using each other's presence, abundance, or phenotype as cues (as investigated empirically by Phillimore et al. (2012) with butterflies and their host plants), rather than using a unique climatic variable such as temperature alone. Second, the expression of maladaptive responses to an unreliable cue could be reduced through learning, for example if females are able to recalibrate their cue use based on past experience (Grieco et al., 2002).

3.2 Consequences on the expression and evolution of plasticity

It is difficult to predict in general whether and how much plasticity should be adaptive in novel environments. It has been suggested that reaction norms should have random and erratic shapes in environments that were never or rarely encountered previously (as is expected to occur under global change), due to the absence of past selection in these environments (Ghalambor et al., 2007). However, more quantitative arguments can be made by taking into account two realistic features of plasticity in new environments. First, environments are seldom entirely new; instead, major environmental shifts mostly mean that previously (perhaps very) rare environments have become common. How rare a new environment has been prior to the shift determines how relaxed selection has been in this environment, and the opportunity there has been for genetic drift to produce erratic reaction norms there (Chevin and Hoffmann, 2017). Second, reaction norms do not evolve completely freely, even in environments where they have not been under selection, because their shapes are generally constrained to some extent by genetic correlations of trait values across environments. Considering these two points makes it likely that reaction norms remain at least partly adaptive over the new environmental range in the absence of major changes in the adaptive landscape, and simple quantitative statements can be made about this question based on the frequency of extremes and the correlations of trait values across environments (Chevin and Hoffmann, 2017).

Retaining partially adaptive plastic responses in novel environments also requires that cues remain reliable. In the specific case of extreme climatic events, whether such cues exist is still unknown. One recent study tackling this issue found no evidence that the Eurasian oystercatchers (*Haematopus ostralegus*) uses cues (lunar cycle, past and current water height) to avoid flooding of their nests during extreme precipitation events (Bailey et al., 2017). Furthermore, global change generally involves variation of more than one environmental variable, and an alteration of the correlation structure of these variables within and between times. This may trigger conflicting ecological demands on organ-

isms (Valladares et al., 2007), such that new combinations of environmental variables become the best predictors of the optimum phenotype. The ability of organisms to track these altered patterns of change in the optimum phenotype is predicted to depend on their ability to evolve a new combination of environmental variables used as a cue (Chevin and Lande, 2015). This theoretical prediction has not been tested yet, but a couple of studies suggest that cue use can evolve relatively fast. A study in the pitcher plant mosquito (*Wyeomia smithii*) suggests that the critical photoperiod for diapause induction has shifted in 5 years towards shorter day length (i.e. later in the season) due to longer growing seasons (Bradshaw and Holzapfel, 2001). Antipredator responses of the Iberian waterfrog (*Pelophylax perezi*) tadpoles to an invasive species (crayfish, *Procambarus clarkia*) is also a likely case of cue evolution. Tadpoles from this species have the ability to build morphological defenses against native predators (Nunes et al., 2014a) but only tadpoles from populations coexisting with *P. clarkia* since 10-15 generations display anti-predator response to this new predator (Nunes et al., 2014b).

Changes in cue reliability are expected to have a major impact on the evolution of plasticity, including a transition between predictive plasticity and bet-hedging as adaptive strategies (Botero et al., 2015; Tufto, 2015). In the context of climate change and the associated increased variability of temperature, decreased predictability of the environment could for example impact the adaptiveness of transgenerational plasticity, and select for a strategy of bet-hedging, where parental effects increase variance in offspring phenotype rather than alter their mean phenotypes (Donelson et al., 2018).

In terms of predictive plasticity, the reliability of the cue determines the steepness of the optimal reaction norm: the less reliable the cue, the shallower the reaction norm that evolves at equilibrium, as compared to “perfect” plasticity, defined by how the optimum phenotype is affected by the environment (Tufto, 2000; Lande, 2009). In the case of an abrupt change in cue reliability, causing previously adaptive plasticity to become maladaptive (for instance because it causes overshoots of the optimum as in Reed et al., 2010), plasticity is expected to evolve towards a shallower reaction norm that matches the current level of cue reliability. Such plasticity shift may cause evolutionary rescue by reducing the load caused by stochastic fluctuations in the environment (Chevin et al., 2013; Ashander et al., 2016).

4. Case study: local variation of cues in Mediterranean blue tits and their preys

Investigating the mechanisms of plastic response to climate change and their limits in the wild is notoriously difficult (electronic supplementary material, Box S1). However, an exceptionally well-characterized research model of adaptive plasticity in wild populations is the earlier egg-laying date of temperate forest insectivorous passerines during warmer springs. This earlier breeding phenology allows birds to track the phenology of the caterpillars they use as

main food source for their offspring (Chevin and Lande, 2015; Visser et al., 2006). In the following section, we investigate in some detail how plastic phenological change relates to environmental cues and their reliability in four Mediterranean populations of blue tits (*Cyanistes caeruleus*).

The four wild populations of blue tits have been monitored for 20 to 42 years (electronic supplementary material, table S1). Two populations breed in forests dominated by the deciduous downy oak *Quercus pubescens* (the D-Rouvière and the D-Muro populations, in the French mainland close to Montpellier and Corsica respectively, where ‘D’ stands for deciduous) and two in forests dominated by the evergreen holm oak *Quercus ilex* (the E-Muro and the E-Pirio populations, both in Corsica; ‘E’ stands for evergreen forest, Blondel et al., 2006). These ecological characteristics have induced local adaptation in birds (Charmantier et al., 2016), particularly in their life-history traits. Average phenology of two of these populations has been changing over time: mean laying date is increasingly early (more than three days per decade) for the populations breeding in evergreen, but not in deciduous forests (figure 2a and electronic supplementary material, table S2a), despite similar trends of climate warming in the four sites (electronic supplementary material, table S2c and figure S1; note that table S1 reports trends in temperature over the spring – from April to end-June – while figure S1 presents trends in temperature over the entire year). This pattern is all the more striking as the E-Muro and D-Muro populations are only 5.6 km apart (Charmantier et al., 2016), and connected by gene flow (Szulkin et al., 2016).

One plausible explanation behind the different time trends in laying date could be local variation of environmental cues for phenotypic plasticity. Females from different populations could be sensitive to different climate variables (e.g. amount of rain, mean temperature or presence of extreme climatic events), or to the same variables but at different time periods during the year (e.g. early vs. late spring). In order to assess the population-specific cues for laying date plasticity in blue tits, we implemented sliding windows analyses using the package *climwin* (Bailey and van de Pol, 2016; van de Pol et al., 2016). For each population, we tested several weather variables: mean, maximum, minimum for both daily temperature and daily rain, and positive Extreme Climatic Events of temperature (ECE hereafter, electronic supplementary material Box S2 for details on the methods). Climate data were obtained from the national meteorological stations of Saint Martin de Londres for the mainland (about 24 Km from D-Rouvière) and Calvi for Corsica (9-19 km from the three Corsican study sites). Temperatures from the meteorological stations are highly correlated to local temperature in study sites (electronic supplementary material, table S3).

In three of the populations (D-Rouvière, D-Muro and E-Pirio), the climatic factor that most influences variation of mean laying date is the mean temperature (electronic supplementary materials, table S4) explaining between 54 and 75% of variability in laying date (table 1a). “Evergreen” populations are also sensitive to the number of ECE. In E-Pirio, this effect is additive with the mean temperature effect, but not highly robust. In turn, the number of ECE

within a window is the best predictor of average laying date in the E-Muro population (electronic supplementary materials, table S4), but the simultaneous use of information from mean temperature cannot be excluded (mean temperature: -3.47 ± 0.88 , $t = -3.94$, $p = 0.001$, ECE: -1.59 ± 0.42 , $t = -3.79$, $p = 0.002$, $r^2 = 0.75$). We found no effect of other climate variables such as rainfall (electronic supplementary materials, table S4). Hence, although some combination of other factors (e.g. bud development, Bourgault et al., 2010; or population density, Bourret et al., 2015) likely play a role, temperature is a key driver behind the plasticity of laying date. This is in line with other correlative studies in great tits, showing that the average spring temperature explains more than 50% of the variability of laying date (Visser et al., 2006; Charmantier et al., 2008), and with experiments showing that laying date responds to temperature treatments (Visser et al., 2009). The underlying physiological mechanisms remain elusive, as no effect of temperature on the neuroendocrine system linked to reproduction has been detected yet (Schaper et al., 2012; Caro et al., 2013a). Despite many studies on bird breeding phenology, knowledge about mechanisms driving phenological plasticity are still scarce, and the relative roles of direct and indirect effects of temperature (e.g. for the latter, a constraint on the timing of egg laying mediated by food abundance or quality) are unknown (Caro et al., 2013b).

The populations differ in the specific time window of mean temperature influencing laying date. Females from populations in deciduous habitats use a cue based on a long time period encompassing winter and early spring, while females from the E-Pirio population use a shorter and later time period corresponding to a month in spring (figure 3, electronic supplementary materials, table S5). The pattern is qualitatively similar but not robust in E-Muro (figure 3, electronic supplementary material, table S4 and S5). Larger windows in deciduous populations could be related to the correlation between the best window and other possible windows remaining high across a larger range of time periods than in evergreen populations (electronic supplementary materials Figure S2). A formal statistical comparison between the windows used in D-Rouvière and E-Pirio shows significant differences between the two populations in terms of ordinal calendar (electronic supplementary materials Box S2).

Table 1 Average population reaction norms in response to the cues estimated with the sliding window analyses (see figure 3, table S5) for a) laying date of blue tits, and b) caterpillar date of peak abundance. Depending on the best predictors of phenology detected by sliding window analysis, the reaction norms were estimated using either the mean temperature (MeanT) or the number of ECE during the best window. Sample sizes are given in the electronic supplementary material table S1.

	Cue	Intercept			Slope			
		Estimate (SE)	t-value	p-value	Estimate	t-value	p-value	
a)							r^2	
D-Rouvière	MeanT (14th Jan. - 1st Ap.)	131.78 (3.82)	34.51	<0.0001	-4.42 (0.50)	-8.91	<0.0001	0.75
D-Muro	MeanT (4th Feb. - 7th Ap.)	137.61 (6.78)	20.31	<0.0001	-3.67 (0.62)	-5.92	<0.0001	0.59
E-Muro	MeanT (15th March - 27th Ap.)	175.33 (13.34)	1.14	<0.0001	-5.13 (1.00)	-5.11	<0.0001	0.57
	ECE (23th March - 7th May)	112.21 (1.21)	92.90	<0.0001	-2.41 (0.49)	-4.95	<0.0001	0.55
E-Pirio	MeanT (31th March - 7th May)	200.53 (11.92)	16.82	<0.0001	-5.10 (0.84)	-6.07	<0.0001	0.54
b)							r^2	
D-Muro	MeanT (11th Feb. - 7th Ap.)	171.22 (9.01)	19.01	<0.0001	-5.00 (0.81)	-6.15	<0.0001	0.61
E-Pirio	MeanT (13th March - 7th June)	282.38 (20.04)	14.14	<0.0001	-8.47 (1.31)	-6.46	<0.0001	0.58

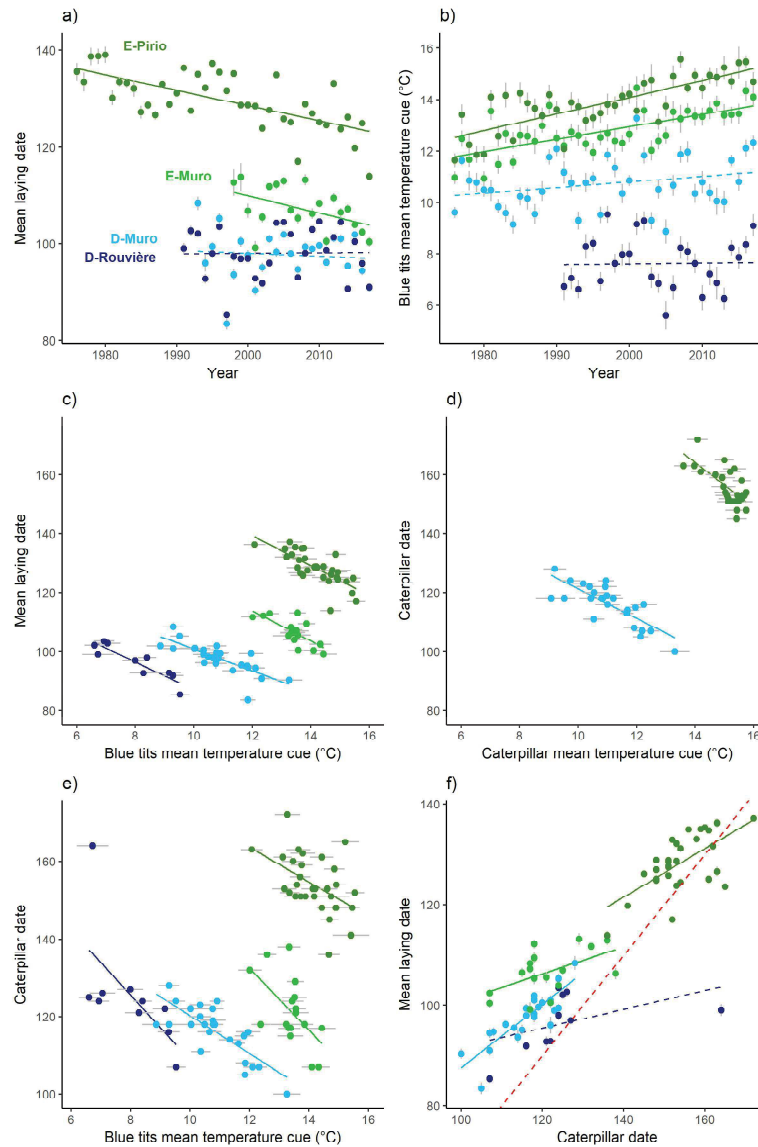


Figure 2: a) Temporal trends in annual mean laying dates in the four Blue tit populations (mean \pm SE); b) Temporal trends in mean temperature (in $^{\circ}\text{C} \pm$ SE) for the cue windows used by each population; c) Blue tit reaction norms: annual mean laying date (\pm SE) as a function of the estimated cue (in $^{\circ}\text{C} \pm$ SE); d) Caterpillar reaction norm (only for E-Pirio and D-Muro): annual caterpillar peak date as a function of the estimated cue ($^{\circ}\text{C} \pm$ SE); e) Cue reliability for blue tits measured as the correlation between the caterpillar peak date and the cue (table S7); f) Synchrony between blue tits and caterpillars illustrated as the correlation between mean laying date of blue tits (\pm SE) and caterpillar peak date (see table S8). In all panels, the “Evergreen” sites are in green (dark green for E-Pirio, light green for E-Muro), and “Deciduous” sites are in blue (dark blue for D-Rouvière, light blue for D-Muro). All dates are ordinal dates with 1= 1st January. In panel f, the red dashed line represents the expected optimal relationship between laying date and caterpillar date. In some cases, error bars are not visible because they are very small compared to the figure scale.

The use of different time windows in the four bird populations leads to contrasted rates of change of the cue over the study period. In the “Evergreen” populations, where the window includes only spring temperature, the mean temperature inside the cue window increases over the 1991-2017 period (E-Muro: 0.4 ± 0.07 °C/decade, t-value = 6.18, p-value < 0.0001; E-Pirio: 0.6 ± 0.08 , t-value = 7.81, p-value < 0.0001, figure 2b), but there is no such increase in the two other sites where the windows include information from spring and winter (D-Rouvière: 0.03 ± 0.3 °C/decade, t-value = 0.11, p-value = 0.91; D-Muro 0.22 ± 0.12 , t-value = 1.76, p-value = 0.09). This is in line with the very slow or absent rate of climate change in winter as compared to spring in these Mediterranean areas (electronic supplementary material, figure S1). Climate change is also associated with increasing frequency of ECE, and their number increases in the window used by the E-Muro population (0.49 ± 0.16 positive ECE/decade, t-value = 2.98, p-value = 0.005, see electronic supplementary material table S6 for other populations). Hence overall, even though spring warming has been pervasive in all populations (electronic supplementary materials, table S2c, figure S1), we only detect climate warming during the cue windows influencing bird phenology in E-Pirio and E-Muro. This difference of windows across habitats explains laying dates are advancing in the evergreen avian populations but not in the deciduous populations.

It is also possible to investigate the reliability of cues in this system. The fitness consequences of phenological responses of blue tits depend on the phenology of caterpillars of *Tortrix viridana*, the main food resource for nestlings (Bańbura et al., 1994), thought to influence reproductive success through offspring survival. The synchronization of the nesting period (especially when nestlings reach the peak of their energetic needs, around 9 days after hatching Dias and Blondel, 1996) with date of peak abundance in caterpillars depends on how reliably the environmental cue used by birds to modify their phenology predicts caterpillar peak date. We can thus measure the linear regression slope of the environment affecting selection on timing of breeding on the environment affecting timing of breeding itself, in order to quantify the reliability of the cue used by birds in a way that matches theoretical predictions (e.g. Gavrillets and Scheiner, 1993; de Jong, 1999). Caterpillar phenology is studied in the four sites thanks to coprometers (i.e. 50*50 cm cloth square collecting the frass of caterpillars under oak trees). The mass of frass collected during a given time period allows us to estimate the abundance of caterpillars throughout the season. The peak date of caterpillar abundance is the day with the highest collected quantity of caterpillar frass. To evaluate the cue used by caterpillars, we used the same sliding window analysis as for birds. Dataset sizes allow caterpillar sliding windows analyses only in E-Pirio and D-Muro (at least 20 years of data are required van de Pol et al., 2016). Temperature explained ca. 60% of the variation in the date of caterpillar peak in both sites (table 1b, figure 3, electronic supplementary materials table S5). The reliability of the cue was high in both populations: 1.02 ± 0.04 (t-value = 25.17,

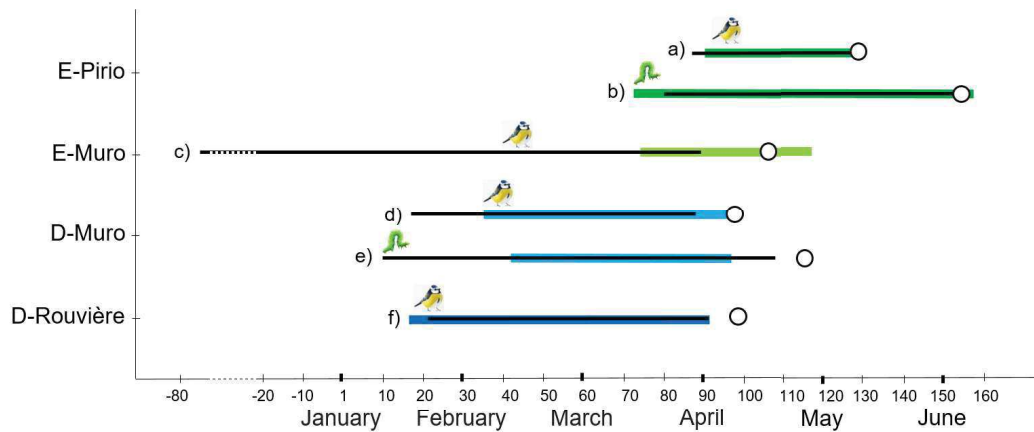


Figure 3: Estimated mean temperature cue windows for blue tits (a, c, d, f) and caterpillars (b, e) in each population. The open circles represent the mean phenology (mean laying date or mean caterpillar peak date). Open and close day of the window are given in ordinal day, with 1 = 1st January. Coloured lines represent the best windows (from the best model, green for evergreen and blue for deciduous); black lines represent the median windows from the 95% confidence set (see electronic supplementary material, Box S2 and Bailey and van de Pol, 2016; van de Pol et al., 2016). A good agreement between best and median window is an indicator that the window is precisely estimated.

p-value < 0.0001) in D-Muro and 0.70 ± 0.08 in E-Pirio (t-value = 9.071, p-value < 0.0001; with lower reliability in E-Pirio than D-Muro (negative blue tit cue*E-Pirio interaction: -0.32 ± 0.09 , t-value = -3.62, p-value = 0.0005).

Another quantity of interest is how well the cue used by birds predict the food peak itself. The slope of the regression of the caterpillar date on the cue used by blue tits is the reaction norm that is favoured by natural selection, if caterpillar date is taken as the optimum phenotype each year. This relationship is significantly negative in all sites (figure 2e, electronic supplementary material, table S7): early prey abundance is associated with high temperature in the cue window. However, the proportion of variation in the food peak that is captured by its relationship with the cue for plasticity is lower in E-Pirio ($r^2 = 0.21$) than in D-Muro ($r^2 = 0.59$, see discussion), and also moderate in the two other populations (D-Rouvière: $r^2 = 0.33$, E-Muro $r^2 = 0.29$). The strength of this relationship in D-Muro is similar as that found for a Dutch great tit population in Hoge Veluwe (Visser et al., 2006). If the food peak correctly predicts the optimum laying date, this suggests that plasticity in response to temperature in the time windows we identified only allows tracking a moderate proportion of temporal fluctuations in this optimum. However, note that caterpillar abundance data in the D-Rouvière site should be used with caution: data collection stopped in 2002 because of frequent rainfalls, and 10 years of data may not be sufficient for analyses to be reliable.

A plasticity-mediated phenological mismatch between predators and their food source can arise from use of different cues, or from different responses to the same cues. Regardless of the mechanism, theory predicts that the slope

of the consumer should be shallower than the slope of the resource, because cue reliability is expected to be lower in consumers (Gienapp and Brommer, 2014). Accordingly, a wide scale analysis over 812 taxa revealed that secondary consumers have lower climate sensitivity than other groups (e.g. primary producers or consumers Thackeray et al., 2016). In line with expectations, in E-Pirio birds are less sensitive to temperature than caterpillars (figure 2c,d, table 1, species*temperature interaction: -4.06 ± 1.28 , t-value = -3.17, p-value = 0.002). However, in the D-Muro site, the slopes of the reaction norms are similar in birds and caterpillars (figure 2c,d, table 1, species*temperature interaction: -1.32 ± 1.02 , t-value = -1.29, p-value = 0.20). The abundance of caterpillars in D-Muro is (much) higher than in the three other populations (electronic supplementary material, table S1). This makes it likely that in other sites such as E-Pirio, birds are exploiting other resources, especially when caterpillars are rare (Bañbura et al., 1994; Tremblay et al., 2005). When food sources are diversified, flatter reaction norms in birds than in caterpillars may not imply that the reaction norms are not adaptive, but that birds are responding to a more multidimensional environment (Chevin and Lande, 2015). An important next step would thus be to estimate how the cue for avian phenology and the date of caterpillars jointly predict the annual optimum laying date (Chevin et al., 2015). However, this may not be sufficient to get a full understanding of the role of plasticity in adaptation in this system. For example, even in D-Muro where average laying date is tracking the caterpillar date accurately, birds are always late compared to the food peak (figure 2f, electronic supplementary material table S8), and there is strong directional selection for earlier laying date (Porlier et al., 2012). An integrative estimate of optimal laying date, using multiple components of fitness such as survival or recruitment success (as in e.g. Price et al., 1988; Reed et al., 2013), could help understand this lag, and whether or not it is adaptive.

5. Discussion and perspectives

Our literature review and case study make it clear that, if we wish to understand how global change will affect phenotypic plasticity and its consequences for population biology, we need a deeper understanding of the environmental mechanisms shaping variation in plasticity within species.

Perhaps the most difficult and needed measurements concern spatio-temporal changes in phenotypic selection, which underlie the evolution of phenotypic cues for plasticity. There have been repeated calls for measuring the environmental sensitivity of selection, notably as a way to identify the causes of natural selection (Chevin et al., 2010; Wade and Kalisz, 1990; MacColl, 2011). This becomes a necessity when investigating the adaptiveness and evolution of plasticity, but the available methods and their applications are still limited (Chevin and Lande, 2015; Hunter et al., 2018). In particular, we are not aware of an attempt to use multiple environmental variables as predictors of changes in an optimum phenotype, as a way to investigate selection on environmental cues for plasticity, consistent with predictions from theory (Gavrilets

and Scheiner, 1993; Chevin and Lande, 2015). Manipulative experimental approaches are also a powerful yet underused tool for measuring selection on phenotypic plasticity and understanding the adaptive role of environmental cues. For instance, Schmitt et al. (1995) experimentally shut down plastic stem elongation in response to crowding in plants, by constructing genetically modified lines of *Brassica rapa* that lack the photoreceptor phytochrome A involved in the detection of the relevant cue (ratio of wavelength characteristic of shading by other plants). They showed that the resulting lack of plastically induced elongation is detrimental in a crowded environment, while constitutive expression of stem elongation is detrimental in uncrowded environments. Other experiments of this kind, where cue perception/use is disrupted, or decoupled from the selective pressure, would yield extremely useful information, but are still too rare.

Furthermore, evaluating whether phenotypic plasticity will remain adaptive in the face of global change requires an integrative approach at different levels. The first level of integration is across life stages. Environmental changes can affect phenotypic expression at different stages of the life cycle, so that the effects of global change can be mediated not only by current cues, but also by transgenerational, early-life, and carry-over effects (figure 1). The investigation of how these mechanisms of response to the environment interact is just starting (Donelson et al., 2018; Beaman et al., 2016), but it will provide important results on the dynamics of expression of plasticity. Second, there is also a strong need for an integrative approach at the community and/or ecosystem level. Interactions among species are a major source of selection for adaptive plasticity (e.g. synchrony of phenology, antipredator defences, competitive interactions). For example, because the migrant pied and collared flycatchers display shallower reaction norms to temperature than blue tits, they breed later in warmer environments, and suffer from competition with resident tit species (Samplonius et al., 2018). An important step would be to increase the number of studies comparing plasticity of interacting species in wild populations, notably examining how climate and environmental change are affecting cues and the expression of plasticity across trophic levels.

Comparing populations can provide insights as to how phenotypic plasticity and cue use are shaped by evolution. In our case study, geographically very close, and connected, populations occupying different habitats differed extensively in the cues affecting laying date plasticity, as well as the reliability of these cues as predictors of the putative selective pressure, namely the peak of abundance of the main food source (caterpillars). The populations from evergreen and deciduous habitats differed in the time windows – and to a lesser extent, in the environmental variables – that they use. The difference in time windows among populations is consistent with results from another study showing shorter windows for populations breeding in “late” habitats, such as high latitudes (Phillimore et al., 2016). This suggests that these differences in time windows can be adaptive. However, detection of cues in wild populations relies on a correlative approach, and we cannot completely exclude that another unmeasured environmental factor correlated to temperature drives the plastic-

ity of laying date, as well as local variation of cue windows. The ecological reality of these populations is certainly highly complex, and more information on environmental heterogeneity would be needed to assess which aspect of environmental variability can be linked to the difference in cue use. For instance, the focal populations vary in the abundance of caterpillars, suggesting that the required use of additional preys may partly explain the apparent difference among populations in the reliability of cues for predicting the selective environment. Altogether, understanding the phenology of blue tits (and other insectivorous passerines in temperate forests) requires integrating knowledge on – at least – three trophic levels: an insectivorous bird, a herbivorous caterpillar and an autotroph tree. In temperate regions, spring temperature seems to be the main environmental factor influencing hatching and development period in insects (van Asch and Visser, 2007), following a period of low metabolic rates caused by ambient temperature during winter (or even a diapause period mainly driven by photoperiod Tauber and Tauber, 1981). Similarly, tree phenology often includes a chilling period (i.e. a period of cold temperature required to lift dormancy after winter Polgar and Primack, 2011). Chilling requirement and cold tolerance vary among species, more specifically deciduous oaks seem more sensitive to cold temperatures and freezing stress than evergreen oaks (Cavender-Bares and N.M., 2001; Cavender-Bares et al., 2005). Cold sensitivity of deciduous oaks suggest that winter temperature could partly predict tree and thus insect phenology, offering a potential explanation as to why winter temperatures are included in the cue window of birds breeding in deciduous oak forests. Finally, the use of extreme climatic events as an additional source of information in E-Muro (ECE) also requires further investigation before clear interpretations can be made. Our results overall highlight that ecological (biotic and abiotic components) variation can have strong effects on plasticity, but further empirical work is needed to be able to discuss our results from a more mechanistic point of view.

We find little evidence for multiple redundant climatic cues, but we cannot exclude that some other, unmeasured cues, such as tree phenology, could also be important in determining laying date (Bourgault et al., 2010). Although partial redundancy should increase the reliability of the information (Dore et al., 2018), information acquisition is also expected to be costly (Dewitt, 1998). An open remaining question is thus the circumstances under which we can expect organisms to use several, partly redundant sources of information, and whether this could mitigate the effects of global change. For example, while temperature in late winter/early spring may not remain a reliable cue, tree phenology could provide more robust information on the long term. Detecting redundant environmental cues is challenging because of collinearity among factors leading to statistical issues (as is probably the case in our analysis), especially in short time series (but see Morrissey and Ruxton, 2018).

If a cue is no longer informative, there could be evolution of cue use. In general, we know little about the evolutionary potential of cue use but a couple of studies suggest some genetic variation exists for this. For example, in *Daphnia magna*, the integration of different cues for plasticity of life history traits

varies among clones (Harney et al., 2017). In *A. thaliana* the knowledge of genetic pathways involved in determining flowering time allows to understand variability in plastic responses and how multiple cues are integrated, and thus to predict the effects of environmental changes on plasticity (Wilczek et al., 2010, 2009). Similar approaches in the wild seem very challenging for now, but laboratory experiments could provide very useful results in this yet little explored area.

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Ethics

The blue tit monitoring protocol was approved by the Animal Care and Use committee Languedoc-Rousillon (CEEA-LR-12066) as well as by Regional Institutions (bylaw issued by the Prefecture on 15/06/2012 n° 2012167-0003). Captures of breeding birds were performed under ringing permits delivered by the CRBPO (Centre de Recherches sur la Biologie des Populations d'Oiseaux, Paris; ringing permit number 1907 to AC, program permit number 369).

Data accessibility

Data will be available from the Dryad Digital Repository after acceptance of the article.

Meteorological data are accessible from <https://donneespubliques.meteofrance.fr/>

Author's contributions

C.T., A.C., L.M.C., S.B. designed the research. A.C., C.T. and S.B. (and many other contributors) collected field data. S.B. conducted statistical analyses. C.T., L.M.C., A.C., and S.B. wrote the paper.

Competing interests

We have no competing interests.

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Supplementary materials

Box S1: Evaluating cues in the wild / in experimental studies

Plasticity cues can be very difficult to identify in empirical systems. By directly manipulating the environment, experimental work can determine which specific cue triggers phenotypic expression (e.g. defenses induced by predator “smell”, or kairomone [1]). A particular advantage of such laboratory experiments is that they allow cues to be identified by decoupling them from (i) other environmental variables that may covary with them in natura, and (ii) other environmental variables that drive selection on the plastic trait. For example, experimental work on shade responses in plants allowed disentangling the effects of spectral quality (red/far red ratio, an indicator of the presence of competitors) and the amount of light (photosynthetically active radiation, signaling neutral shade e.g. from a rock [2]). However, adequate manipulation of the cue may be more complex than anticipated, because the timing and pattern of variation in the environment is also important in eliciting phenotypic responses. For example, laying date in a Dutch great tit population is more sensitive to patterns of temperature than to average temperature per se [3,4] and so are thermal reaction norms of parasitic wasps [5]. Failing to integrate environmental variability in experiments may lead to poorly evaluate responses to an environmental factor [6].

In nature, in contrast, the difficulty is to assess which environmental variable is actually used by the organisms. This requires long term (≥ 20 years [7]) correlative studies. Many studies of phenotypic plasticity in the wild use a priori knowledge [7], others evaluate which environmental variable explains the highest proportion of phenotypic variance. For climatic predictors (e.g. in studies of phenology), the most common method to achieve this is to use sliding window analyses. But until recently and the development of statistical packages such as climwin [7,8], there was no formal statistical framework to assess the validity of detected cues. The recent methodological developments will allow assessing cues in the wild with more confidence, and will facilitate comparisons across studies. However, combining both experimental and in situ correlative approaches is required for a better understanding of each system. For example, an effect of mean temperature on laying date in great tits in the wild is known, but experimental work in the lab revealed later laying date in captivity than in the wild, suggesting the existence of other cues [9].

While organisms respond to complex environments that combine several environmental variables, a majority of studies only investigate one variable as a potential cue [7]. The use of simultaneous cues is extremely difficult to evaluate for wild populations, as this requires not only to define a set of potentially relevant variables that can be used as cues, but also to have access to long term data for a larger panel of environmental variables. These approaches are also very demanding in terms of power for data analysis, and are still rarely done in the wild (but see [10]).

Table S1

Data summary, detailing for each population: forest type, time span over which data are available for blue tit and caterpillar phenology, and sample sizes for individual laying dates. The phenology of caterpillars is approximated by caterpillar frass mass (in mg/ m^2 /day) collected regularly in coprometers, such that the day with the highest quantity of frass in each year corresponds to the date of the peak of caterpillar abundance of the year [11].

	D-Rouvière		D-Muro		E-Muro		E-Pirio	
Forest type	Deciduous		Deciduous		Evergreen		Evergreen	
Nb. mean laying date obs.	27		25		20		42	
(year span)	(1991 - 2017)		(1993 - 2017)		(1998 - 2017)		(1976 - 2017)	
Nb. females / nb. obs. of laying date	905/2040		752/1433		322/796		884/2120	
Nb. caterpillar date obs.	10		25		17		31	
(year span)	(1991 - 2002)		(1993 - 2017)		(2001 - 2017)		(1987 - 2017)	
Caterpillar abundance (se)	60.23		2069.08		517.86		183.04	
	(8.33)		(447.70)		(94.30)		(23.63)	

Table S2

Temporal trends in the four study sites for a) blue tits (laying date, in days per decade); b) caterpillars (date of peak abundance, in days per decade) and c) temperature (daily mean, daily maximum and daily minimum, in °C per decade) during the reproductive period (from April to end of June) estimated over the period 1991-2017. Significant trends (p-value < 0.05) are in bold. Sample sizes are given in Table S1.

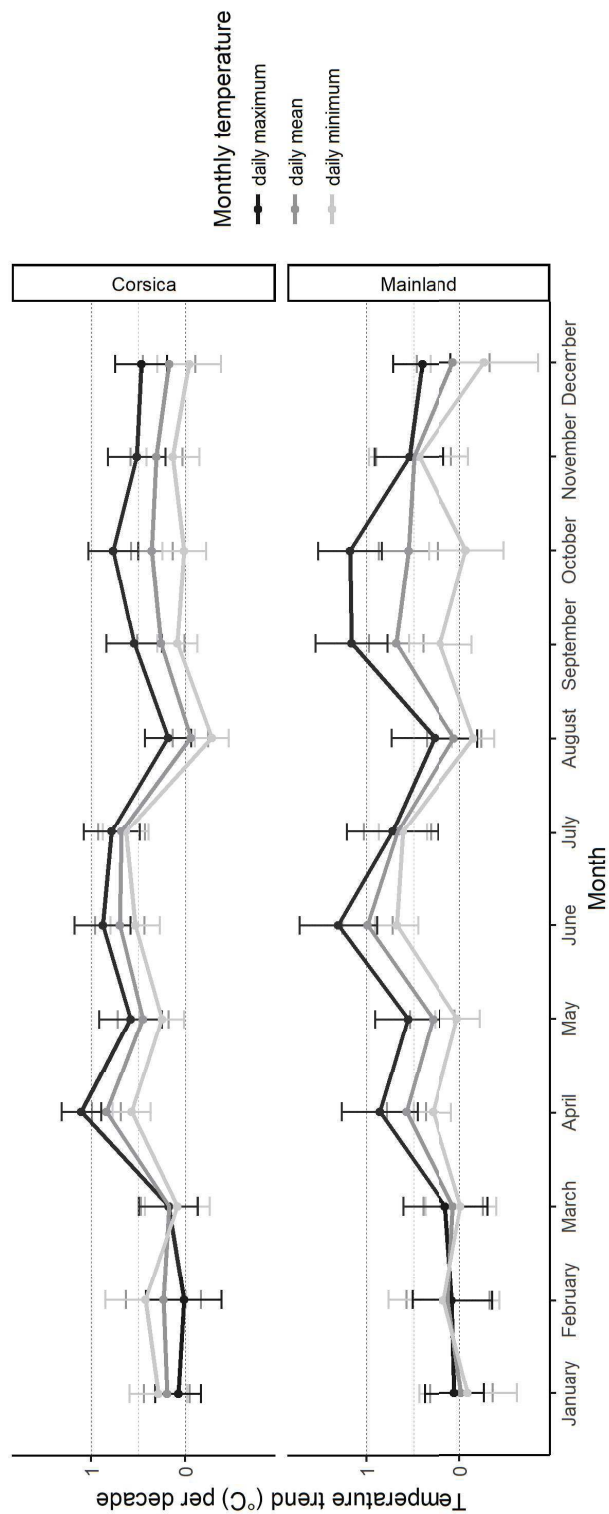
a)	Laying date change (se)	t-value	p-value	r^2
D-Rouvière	0.10 (0.13)	0.08	0.94	-0.04
D-Muro	-0.61 (0.14)	-0.43	0.67	-0.04
E-Muro	-3.57 (0.16)	-2.23	0.04	0.17
D-Muro	-3.15 (0.05)	-5.91	<0.0001	0.45

b)	Food peak change (se)	t-value	p-value	r^2
D-Rouvière	-20.03 (11.51)	-1.76	0.12	0.19
D-Muro	-2.99 (1.80)	-1.66	0.11	0.07
E-Muro	-8.33 (3.93)	-2.12	0.05	0.18
E-Pirio	-3.57 (1.35)	-2.65	0.01	0.17

c)	Statistic	Temperature change (se)	t-value	p-value	r^2
Corsica	Mean	0.66 (0.2)	3.93	0.0005	0.36
	Min	1.03 (0.3)	2.96	0.0067	0.23
	Max	1.25 (0.3)	3.80	0.0008	0.34
Mainland	Mean	0.61 (0.2)	3.92	0.0006	0.36
	Min	0.91 (0.4)	2.55	0.0172	0.17
	Max	1.10 (0.4)	2.68	0.0128	0.19

Figure S1

Time trends of the daily mean (in dark grey), daily minimum (in light grey) and daily maximum (in black) temperature per month across years for Corsican sites (D-Muro, E-Muro and E-Pirio) and the mainland site (D-Rouvière) for the 1991-2017 period. Values are slopes of a regression of temperature against year as a linear trend, estimated for each statistic (mean, max and min) for each month (\pm se).



Box S2

General procedure

In order to estimate the period during which climate most strongly influences the phenology of blue tits and caterpillars, we implemented sliding window analyses using the package `climwin` [7,8]. Correlation between mean laying date or caterpillar date with temperature and rainfall was tested across years for every period of more than 1 day in all years. For mean laying date of blue tits, data on known second clutches were removed, and individuals with laying dates later than 30 day after the first laying date of the year in each population were eliminated from the data sets. We tested several summary statistics for both temperature and rainfall: daily mean, maximum, minimum temperature during each period for all populations. We defined Extreme Climatic Events (or ECE) as the highest 5% of the distribution of a climatic variable over a period of reference [12]. We chose this reference to be the period of sensitivity to mean temperature (population specific best model, see below) for the years 1991-2017. For each climatic parameter, we tested for both linear and quadratic reaction norms. We allowed the window to overlap with the period of reproduction and development (respectively for birds and caterpillars), as a way to assess whether plasticity is predictive, i.e. if the best cues are found before and not during the breeding season.

Among all tested combinations of climate parameters, summary statistics, and relationship functions, we retained the models that best predicted the among-year variation in the mean phenotype for each population and species. Model choice was based on three indicators of the ability of the models to explain the among-year variation of the phenotype: i) The ΔAIC is the difference between the Akaike Information Criterion (AIC) of a given model and that of a basic model including an intercept only, hereafter the base model. This difference estimates the ability of the focal model to explain the temporal variance in the mean phenotype, as compared to the base model. The lower the ΔAIC , the higher the explicative power of the detected cue windows. ii) The critical probability P_c represents the probability to detect the best windows as a signal just by chance (akin to a p-value). We ran randomization procedure to test for the probability of false positives. We ran five simulation models using different randomizations of the mean phenotype across years (five randomisations are sufficient when using P_c according to robustness analyses of van de Pol et al. [7] for each type of climate variable and summary statistics. The best model in these simulations with randomized datasets was chosen based on ΔAIC , and the probability of false positive in cue detection was then quantified as P_c . The smaller the P_c , the lower the probability that the cue window is detected just by chance. iii) The confidence set (%CI), quantifies the extent to which there are several models with very similar explanatory power, and thus provides a confidence interval on window limits. It is the percentage of all tested window models (for a given combination of climatic variable and summary statistics) that are required to reach 95% of the sum of all Akaike weights ($wAIC = \exp(\Delta AIC)$). The models within this set compose the 95%

confidence set of windows for the particular climatic variables tested. A small %CI suggests that only a few window models contribute to the cumulative predictive power of all tested windows, such that the best window is a reliable signal. The smaller the %CI, the higher the reliability of the detected cues windows (opening and closing day of the windows are detected precisely).

The best model is the one that combines the smallest ΔAIC , the smallest %CI and the smallest P_c . In small datasets with lack of statistical power, %CI will increase. A window could have the best ΔAIC but a high P_c , meaning that the signal, despite strong statistical explicative power, is likely to be detected just by chance. In such a case, we evaluated the second best ΔAIC ; if the latter was associated with high P_c , we moved on to the third best ΔAIC , etc, and selected the first model in the list with low P_c . In small data sets, windows length will be sub-estimated [7]. For the summary statistic that best explains among-year variation of the phenotype, we estimated the median windows of models in the confidence set at 95%. Note that mean temperature and the number of ECE are significantly positively correlated for blue tit windows in D-Rouvière ($r^2 = 0.68$), D-Muro ($r^2 = 0.48$) and E-Pirio ($r^2 = 0.26$), but not in E-Muro (p-value = 0.09, $r^2 = 0.12$).

Comparison of windows between populations

We wanted to test if the differences in best cue windows of blue tits from D-Rouvière and E-Pirio were significant. We focused only on these two populations because statistical power was high and equivalent in these populations, but much lower in the two other D-Muro and E-Muro populations. We computed the $\Delta AIC_{\text{same windows}}$ of a model where a single window was estimated for both populations. This was done by running the analysis on a pooled dataset including both the D-Rouvière and E-Pirio populations, with a fixed effect for population, in interaction with climate (i.e. mean temperature in window). Interactions between population and climate effects in the base line model allowed estimating different reaction norms of plasticity of laying date with respect to temperature for each population, but still using the same cue window for both populations. We then compared this model to the sum of ΔAIC of the best models (models 1) of D-Rouvière and E-Pirio estimated separately ($\Delta AIC_{\text{different windows}}$). Note that when the two populations are analyzed separately, compared to a model with no population effect, two additional parameters are estimated (open and close days for the second population). We thus added 2 points of ΔAIC in $\Delta AIC_{\text{different windows}}$ compared with the model ($\Delta AIC_{\text{different windows}}$). If the sum of $\Delta AIC_{\text{different windows}}$ is lower than the $\Delta AIC_{\text{same windows}}$, then estimating two different windows for the two populations provides a better fit to the data than a single common window, providing evidence that populations differ in their climate windows.

For comparison, the three sliding windows analyses are implemented with the same reference day (starting day of the sliding windows) equal to 1st July (however, results are consistent regardless of what reference day is used). Results from the sliding windows analyses show that,

$$\begin{aligned}\Delta AIC_{\text{different windows}} &= \sum AIC = \Delta AIC_{\text{D-Rouvière}} + \Delta AIC_{\text{E-Pirio}} + 2 \\ &= -35.44 - 39.08 + 2 \\ &= -72.52\end{aligned}$$

and

$$\Delta AIC_{\text{same windows}} = -19.98$$

This clearly indicates that D-Rouvière and E-Pirio have different opening and closing days of temperature windows.

Table S3

Consistency of information from local records of temperature (iButtons with temperature log every hour) and data from meteorological stations. Data from iButtons are available from 2009 to 2016 in D-Rouvière, and from 2013 to 2016 in E-Pirio, E-Muro and D-Muro. The congruence was estimated by regressions of the local average daily temperature (minimum daily temperature + maximum daily temperature / 2) from i-buttons placed near nest-boxes over corresponding mean daily temperatures from national meteorological stations (meteorological stations of Calvi for Corsican sites and Saint Martin de Londres for the D-Rouvière site). r^2 is the coefficient of determination. Significant slopes (p-value < 0.05) are in bold. Sample sizes are given in Table S1.

	Intercept			Slope			r^2
	Estimates (se)	t-value	p-value	Estimates (se)	t-value	p-value	
D-Rouvière	4.66 (0.40)	11.68	<0.0001	0.84 (0.02)	37.68	<0.0001	0.65
D-Muro	1.85 (0.39)	4.73	<0.0001	0.91 (0.02)	40.92	<0.0001	0.73
E-Muro	0.80 (0.46)	1.76	0.08	0.98 (0.02)	40.24	<0.0001	0.78
E-Pirio	-0.26 (0.25)	-1.02	0.31	0.96 (0.01)	68.19	<0.0001	0.84

Table S4

Results from sliding windows analyses for blue tits and caterpillars. The best models are highlighted in red for each population. ΔAIC : Difference between the Akaike information criteria of the focal model and that of the base model (a model including the intercept only, see electronic supplementary material Box S2 for details about ΔAIC , P_c and %CI). Each line presents the best model from a sliding window analysis with the corresponding climate factors, statistics and function. Open and close day of time windows are counted backwards, starting from the reference day (= earliest date of phenotype + 30 day), consistent with the output format of the sliding windows implemented in climwin [7,8]. Note that in the main text, results are transformed to be expressed forward, with a reference Day 1= 1st January (see also table S5). Sliding windows analyses 1 to 12 and ECE were implemented with the described combination of climate variable, summary statistics and reaction norm function. In the specific case of models 'Cue1 + ECE', sliding windows analyses were implemented including in the base model the best temperature window estimated in model 1, in order to take into account the effects of annual mean temperature. However, in the table, we report for these 'Cue1 + ECE' models the global ΔAIC i.e. the sum of the ΔAIC of the model 1 and of the model 'Cue1 + ECE'. The ΔAIC of all models in the table are thus directly comparable (with reference to a base model with intercept only).

Blue tits														
Parameters														
Model	Cue	Statistic	Function	D-Rouvière				D-Muro						
				Open	Close	ΔAIC	P_2	%CI	Threshold	Open	Close	ΔAIC	P_1	%CI
1	Temperature	Mean	Lin	98	24	-35.44	0.0007	2	-	78	16	-16.84	0.002	6
2	Temperature	Max	Lin	46	46	-11.37	0.52	77	-	49	48	-14.13	0.45	69
3	Temperature	Min	Lin	46	34	-22.54	0.0007	3	-	49	49	-12.60	0.47	75
4	Temperature	Mean	Quad	99	23	-35.12	0.002	2	-	4	1	-17.22	0.05	8
5	Temperature	Max	Quad	77	69	-11.25	0.90	71	-	289	276	-15.88	0.996	47
6	Temperature	Min	Quad	45	34	-21.26	0.002	3	-	4	2	-20.48	0.05	20
7	Rain	Mean	Lin	350	350	-8.60	0.90	93	-	199	195	-15.07	0.73	83
8	Rain	Max	Lin	350	350	-8.60	0.89	91	-	155	155	-13.91	0.55	81
9	Rain	Min	Lin	161	159	-9.66	0.82	94	-	155	155	-13.91	0.94	94
10	Rain	Mean	Quad	292	292	-12.20	0.83	82	-	348	348	-22.75	0.009	9
11	Rain	Max	Quad	351	350	-12.86	0.95	85	-	348	348	-22.75	0.002	14
12	Rain	Min	Quad	350	350	-12.13	0.82	94	-	348	348	-22.75	0.93	87
ECE	Temperature	Sum	Lin	13.36	40	-7.91	0.37	67	-	53	24	-5.72	0.58	77
Cue 1 + ECE	Temperature	Sum	Lin	-	-	-	-	-	-	-	-	-	-	-
Parameters														
Model	Cue	Statistic	Function	Open	Close	ΔAIC	P_2	%CI	Threshold	Open	Close	ΔAIC	P_1	%CI
1	Temperature	Mean	Lin	55	12	-14.92	0.04	41	-	54	17	-39.08	7.5 e-05	1
2	Temperature	Max	Lin	52	14	-14.29	0.13	49	-	28	28	-22.46	7.6 e-05	10
3	Temperature	Min	Lin	310	310	-8.99	0.75	90	-	28	28	-22.46	0.1	56
4	Temperature	Mean	Quad	236	234	-12.77	0.84	52	-	17	54	-37.72	0.001	1
5	Temperature	Max	Quad	32	14	-15.01	0.32	52	-	28	28	-20.10	0.05	20
6	Temperature	Min	Quad	308	299	-11.37	0.84	73	-	28	28	-20.10	0.12	37
7	Rain	Mean	Lin	27	22	-11.35	0.73	92	-	74	8	-18.05	6.2 e-05	6
8	Rain	Max	Lin	24	22	-14.54	0.78	91	-	64	6	-8.66	0.88	84
9	Rain	Min	Lin	175	174	-5.92	0.74	94	-	111	107	-10.18	0.94	94
10	Rain	Mean	Quad	24	17	-15.46	0.87	62	-	24	38	-19.21	0.001	7
11	Rain	Max	Quad	24	19	-17.33	0.24	42	-	250	250	-8.32	0.99	67
12	Rain	Min	Quad	330	330	-5.07	0.74	94	-	64	6	-11.20	0.94	94
ECE	Temperature	Sum	Lin	17.15	24	-18.24	0.002	5	-	50	17	-17.78	0.002	32
Cue 1 + ECE	Temperature	Sum	Lin	17.15	24	-25.84	0.21	62	-	50	33	-49.10	0.03	50
Parameters														
Model	Cue	Statistic	Function	Open	Close	ΔAIC	P_2	%CI	Threshold	Open	Close	ΔAIC	P_1	%CI
1	Temperature	Mean	Lin	88	33	-21.17	0.001	7	-	94	8	-24.8	0.0002	2
ECE	Temperature	Mean	Lin	88	57	-15.91	0.005	21	-	41	40	-9.26	0.25	64
Cue 1 + ECE	Temperature	Mean	Lin	78	76	-10.73	0.14	60	-	18	18	-6.45	0.74	82

Table S5

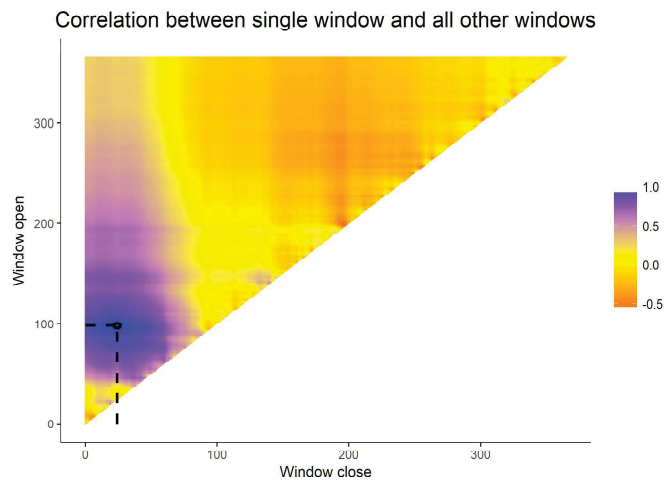
Estimated best and median mean temperature cue windows (see electronic supplementary material Box S2 for details about choice of best windows and estimation of median windows). Ordinal day 1 = 1st January. Negative value of ordinal means day in the previous year (before 1st January).

	Blue tits				Caterpillars			
	Best Window		Median window		Best Window		Median Window	
	Open day	Close day	Open day	Close day	Open day	Close day	Open day	Close day
D-Rouvière	17	91	22	91	-	-	-	-
D-Muro	35	97	19	88	42	97	10	107
E-Muro	74	117	-75	89	-	-	-	-
E-Pirio	90	127	87	130	72	158	80	156

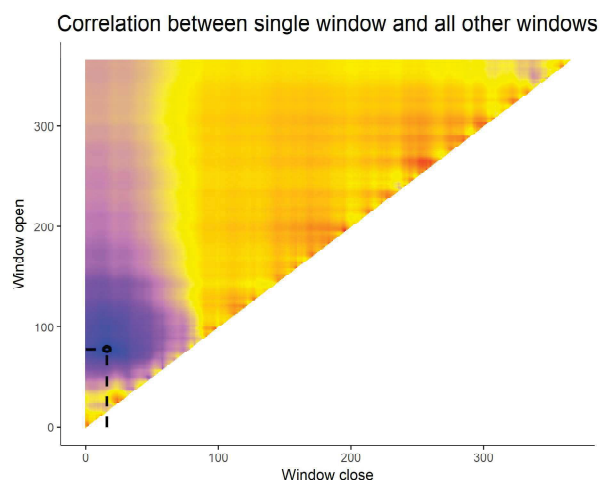
Figure S2

Correlation between the window estimated from the best model (represented by the black dot) and all other possible windows of mean temperature for a) D-Rouvière, b) D-Muro, c) E-Muro and d) E-Pirio population (positive autocorrelation in purple; negative autocorrelation in red). Graphics from the function `autowin` in `climwin` R package. Some differences in patterns of correlation are visible among populations: in deciduous populations the peak of correlation is less steep than in the evergreen populations. This is in line with the interpretation that the environment is less predictable in evergreen populations.

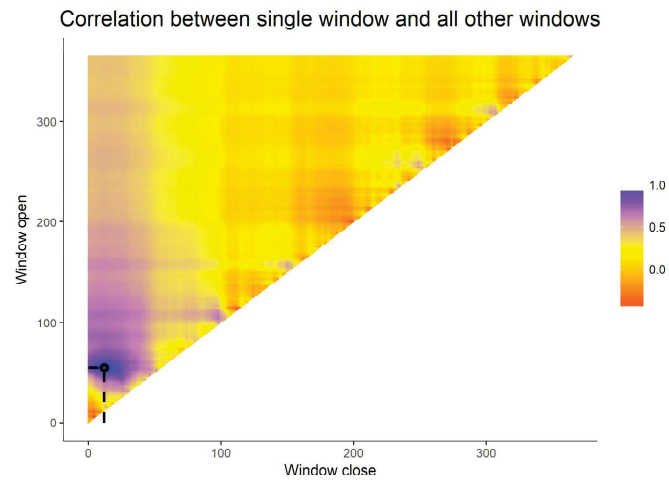
a)



b)



c)



d)

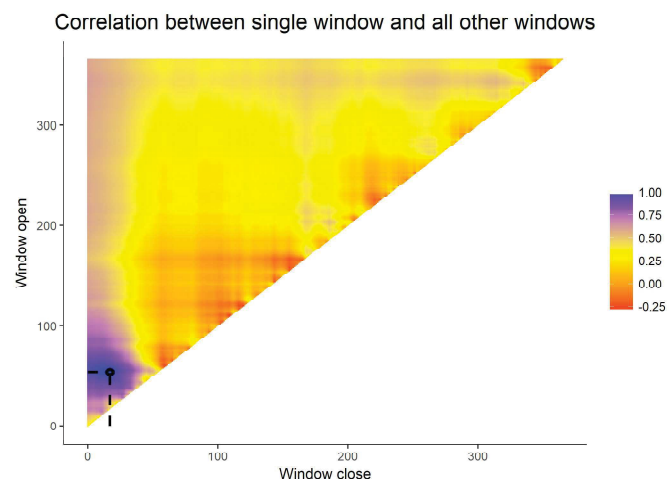


Table S6

Temporal trend in the number of positive ECE at 5% during the cue window (per decade). r^2 is the coefficient of determination. Significant slopes (p-value < 0.05) are in bold. Sample sizes are given in Table S1.

	Number of ECE (se)	t-value	p-value	r^2
D-Rouvière	0.73 (0.71)	1.03	0.31	0.002
D-Muro	0.71 (0.29)	2.45	0.02	0.11
E-Muro	0.49 (0.16)	2.98	0.005	0.16
E-Pirio	1.8 (0.5)	3.48	0.001	0.21

Table S7

Blue tit reaction norm favoured by selection estimated by the regression of the date of caterpillar peak abundance on the detected cues for blue tits. For evergreen populations, we present results for cues taken both as mean temperature (MeanT) and the number of positive ECEs. Models with both MeanT and ECE are not better than models with MeanT (or ECE) alone (not shown). r^2 is the coefficient of determination. Significant slopes (p-value < 0.05) are in bold. Sample sizes are given in Table S1.

	Climate	Intercept			Slope			r^2
		Estimates (se)	t-value	p-value	Estimates (se)	t-value	p-value	
D-Rouvière	MeanT	192.72 (29.17)	6.61	0.0002	-8.39 (3.62)	-2.32	0.049	0.33
D-Muro	MeanT	169.02 (9.02)	18.75	<0.0001	-4.88 (0.83)	-5.90	<0.0001	0.59
E-Muro	MeanT	228.36 (39.36)	5.80	<0.0001	-7.99 (2.93)	-2.73	0.02	0.29
	ECE	125.42 (3.94)	31.81	<0.0001	-1.87 (1.50)	-1.25	0.23	0.03
E-Pirio	MeanT	216.74 (20.87)	10.39	<0.0001	-4.43 (1.47)	-3.01	0.005	0.21
	ECE	162.07 (2.38)	68.09	<0.0001	-0.97 (0.25)	-3.84	0.0006	0.31

Table S8

Synchrony between blue tits and their preys, assessed by the regression between mean laying date of blue tits and caterpillar date of peak abundance. r^2 is the coefficient of determination. Significant slopes (p-value < 0.05) are in bold. Sample sizes are given in Table S1.

	Intercept			Slope			r^2
	Estimates (se)	t-value	p-value	Estimates (se)	t-value	p-value	
D-Rouvière	8.33 (76.56)	0.11	0.92	1.21 (0.79)	1.53	0.16	0.13
D-Muro	7.50 (14.85)	0.51	0.62	1.11 (0.15)	7.32	<0.0001	0.69
E-Muro	8.40 (46.03)	0.18	0.86	1.06 (0.43)	2.45	0.03	0.24
E-Pirio	44.74 (24.38)	1.83	0.08	0.85 (0.19)	4.49	0.0001	0.39

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Fieldwork in Corsica.

(Credits: Anne Charmantier)

Chapter Two

Laying date within-individual variation

MANUSCRIPT B

Age-dependent phenological plasticity in a wild bird

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Abstract

1. Life-history traits are often plastic in response to extrinsic environmental factor(s) (such as temperature or precipitation) and internal factors such as age in many species. Trait variation during the lifetime of individuals could thus be partly driven by age-dependent plasticity of these traits.
2. We study whether plasticity of a phenological trait – the egg-laying date – with respect to spring temperature varies with age and explore its consequences on annual reproductive success of females.
3. We use data from a long term monitoring of a wild population of blue tits in Corsica Island to estimate age-dependent variations of plasticity of reproductive phenology and annual reproductive success.
4. We show that both laying date plasticity and annual reproductive success vary with age: young and old females are less plastic and fledge less offspring than middle-age females. Age-dependent variation of plasticity may have different consequences on reproductive success over female lifetime, as older females may experience lower reproductive success irrespective of their laying date.
5. Phenological plasticity is a major driver of adaptation to rapid environmental variation, our results suggest that understanding its role in adaptation to climate change and population persistence requires integrating the age-structure of the population.

Key words: Phenotypic plasticity, Laying date, Phenology, Age, Blue tit, Wild birds

1. Introduction

Phenotypic plasticity – the capacity of a given genotype to express different phenotypes according to environmental conditions (Pigliucci, 2001) – is a widespread mechanism in nature (DeWitt and Scheiner, 2004). Theoretical and empirical studies highlight the importance of phenotypic plasticity for population persistence (e.g. Chevin et al., 2010; Ashander et al., 2016; Reed et al., 2011). When plasticity is adaptive, it may allow the population mean phenotype to track an environment-dependent phenotypic optimum, thus minimizing the impact of environmental change on mean fitness and population growth. This is especially true for phenological traits, which determine the timing of key life history events such as reproduction, dormancy/diapause, or dispersal, in order to synchronize these events with the optimal timing set by a seasonal – and often also noisy – environment. Phenological traits have been shown to represent a major phenotypic response to rapid climate change (Parmesan and Yohe, 2003; Visser and Both, 2005; Walther et al., 2002; Davis et al., 2005). It is also increasingly clear that plastic changes contribute a large proportion of observed phenotypic change in the wild (e.g. Walther et al., 2002; Gienapp et al., 2007; Merilä and Hendry, 2014; especially for phenological traits, Charmantier and Gienapp, 2014; Vedder et al., 2013).

Life-history traits (e.g. fecundity) are often plastic in response to extrinsic environmental factor(s), and also vary with age in many iteroparous species. Empirical studies commonly show patterns of age-dependent reproductive performances, with increasing annual reproductive success in the beginning of life, followed by maximal reproductive performance in middle-age, and finally, a decreasing success due to reproductive senescence (e.g. Clutton-Brock, 1988; Balbontín et al., 2007; Warner et al., 2016). Since plastic phenology is a strong driver of reproductive performance in many species, notably in birds (e.g. Perrins and McCleery, 1989; Marrot et al., 2018), it is likely that variation of plastic reaction norms with age underlies age-dependent patterns in reproductive success to some extent. If reaction norms vary with age, then understanding the role of plasticity in adaptation to rapid environmental change and population persistence requires integrating the age-structure of the population.

Theoretical studies on the evolution of age-dependent plasticity generally predict a decrease of plasticity with age (e.g. Fawcett and Frankenhuis, 2015; Stamps and Krishnan, 2017; Fischer et al., 2014). However, note that these theoretical models are mostly about binary environments and traits, and/or without within-generation environmental variation, so that formal theoretical predictions are still missing for continuous labile traits under continuous environmental variation.

Within-individual variation of plasticity over lifetime has been highlighted in some experimental studies, mainly in behavioral traits (e.g. Atwell and Wagner, 2014; Ericsson et al., 2016), with highest plasticity in juveniles or young adult followed by decreasing plasticity with age, thus corroborating theoretical predictions. However, evidence is scarce for plasticity variation with age in wild populations. Araya-Ajoy and Dingemans (2016) compared the plas-

tivity of aggressiveness in great tits (*Parus major*) between two age classes, and showed that first year-old breeders were more plastic than older birds, also corroborating theoretical predictions. Overall, the scarce empirical evidence means we still poorly understand the origin of age-dependent plasticity of labile phenotypic traits over lifetime. The study of age-specific variation of plasticity in wild populations is an arduous task because of the combined challenges of studying phenotypic plasticity and senescence (Nussey et al., 2013, 2007a). Any research about plasticity and/or senescence demands long-term study with individual marking and repeated measures on same individuals. In addition, plasticity estimation requires identifying the main environmental cue(s) explaining within-individual phenotypic variation. These challenges explain why age-specific variation of environmentally induced plasticity has been widely neglected so far in empirical studies.

We studied age-specific variation in the plasticity of a phenological trait, the egg-laying date, in a wild Blue tit population. We used linear mixed models to explore the relationship between age-related variation of plasticity and annual reproductive success through female lifetime. In this species, as in most temperate passerines, we observe plastic among-year variation of laying date depending on spring temperature (e.g. Porlier et al., 2012). This variation allows birds to synchronize their reproductive period with the timing of maximal abundance of caterpillars, which are the main nutritive resource for nestlings (Blondel et al., 1991). Annual reproductive performances also vary with age in blue tits: young birds have low annual reproductive success (partially) due to naiveness before learning processes (Gienapp and Brommer, 2014), while old individuals suffer from reproductive senescence (Auld and Charmantier, 2011), leading to quadratic variation of reproductive success with age. Since laying date is a plastic trait in response to temperature, age-related variation of reproductive timing and annual reproductive success may be associated with age-specific laying date plasticity with temperature. We expect age-dependent plasticity patterns to be similar to age-specific reproductive success, and decreasing plasticity with age as predicted by theoretical models (see above).

2. Materials & Methods

2.1 Species and data collection

We used a long-term study of a wild Blue tit (*Cyanistes caeruleus ogliastrae*, a small insectivorous and cavity-nesting passerine) population in the Mediterranean region, initiated by Jacques Blondel in 1975. Breeding data were collected every spring on the study site. The population is on the island of Corsica, in an evergreen forest (E-Pirio population, 42°34'N, 08°44'E) dominated by holm oaks (*Quercus ilex*) (see reviews on the long-term study in Blondel et al., 2006; Charmantier et al., 2016). Blue tits breed in 205 nest-boxes (natural cavities are rare in this forest, Charmantier, unpublished), which were monitored weekly from March to June to record laying date, clutch size and reproductive success. Birds were individually marked with a unique numbered

metal ring. We computed analyses on first broods of the season only (second broods represent less than c. 1% of the clutches). Age (see electronic supplementary material 1 for the number of females in each age class) was estimated based on birth year for individuals born in nest-boxes or on plumage patterns for immigrants. The latter allowed us to distinguish females of one year old versus two years or older. In our dataset, exact age was known for c. 60% of females and minimum age for c. 40%. Similarly, the age at last reproduction of each female may be under-estimated for individuals with unknown exact age, and for all individuals because of imperfect recapture rates of individuals (probability of recapture is 0.8 in this population, Dubuc-Messier et al. *submitted*). The dataset contained 1696 laying date observations from 1976 to 2017, for 854 identified females (electronic supplementary materials 1 and 2). Temperature cue was the mean-centered average daily temperature between 31st March and 7th May, which is the period most correlated with inter-annual laying date variation at the population scale according to sliding windows analyses (Bonamour et al., 2019).

2.2 Statistical analyses

We estimated age-dependent laying date plasticity in response to temperature using the following linear mixed model (e.g. Nussey et al., 2007b),

$$LD_{ij} = \mu + \alpha_1 T_j + \alpha_2 A_{ij} + \alpha_3 A_{ij}^2 + \alpha_4 (T_j A_{ij}) + \alpha_5 (T_j A_{ij}^2) + \alpha_6 ALR_i + ind_i + year_j + \epsilon_{ij} \quad (1)$$

where the laying date LD ($1 = 1^{st}$ January) of female i in year j is explained by several fixed effects: cue temperature T_j of year j , age A_{ij} , and age-squared A_{ij}^2 of female i in year j (with age a continuous variable ranking from 1 to 9 years old). The terms $T_j A_{ij}$ and $T_j A_{ij}^2$ are interactions estimating respectively the linear and quadratic age-dependence of plasticity in laying date with temperature. We controlled for female longevity by adding an effect of age at last reproduction of female i , ALR_i , allowing to model a selective disappearance effect on laying date variation (e.g. Bouwhuis et al., 2009). To take into account the non-independence of data collected on the same female and the same year, we included female identity (ind_i) and year ($year_j$) as random effects. The final term in equation 1 is the residual error term ϵ_{ij} . Male laying date was not repeatable (Auld and Charmantier, 2011), and we thus considered laying date as a female-specific trait.

We estimated the effects of age and laying date on annual reproductive success ARS (approximated as the number of fledgling per breeding attempt) using the following linear mixed model,

$$ARS_{ij} = \gamma + \beta_1 A_{ij} + \beta_2 A_{ij}^2 + \beta_3 LD_{ij} + \beta_4 (A_{ij} LD_{ij}) + ind_i + year_j + r_{ij} \quad (2)$$

where annual reproductive success ARS_{ij} of female i in year j is explained by the female age A_{ij} and age-square A_{ij}^2 , the laying date LD_{ij} and the interaction

between laying date and age ($A_{ij}LD_{ij}$). We included two random effects, female identify (ind_i) and year ($year_j$), and r_{ij} are residuals. Females with clutch manipulation and all other experiments resulting in potential effects on reproductive success were removed from the data set for model 2, reducing the dataset to 1092 laying date attempts for 667 identified females.

All statistical analyses were carried out using the software R (version 3.3.2, R Core Team, 2016), and performed in a Bayesian framework using a Markov Chain Monte Carlo estimation algorithm (R package MCMCglmm Hadfield, 2010). For all random effects and the residual variance, we used a weakly informative prior, corresponding to an inverse-Wishart $W^{-1}(\psi, \nu)$ distribution with ψ equal to the phenotypic variance of laying date divided by the number of estimated variance components. ν is the degree of belief in the prior and we tested the robustness of results to the prior specification using $\nu = 1, 0.2$ or 0.02 . The default Gaussian non-informative prior in MCMCglmm with mean zero and variance 10^8 was used for the fixed effects. Models were run for 1,010,000 iterations, including a burn-in period of 10,000 iterations. We ran three chains which were sampled every 1000 iteration to avoid autocorrelation. The maximum autocorrelation between estimates is < 0.05 for fixed and random effects. Convergence of chains was tested using Gelman and Rubin's convergence diagnostic (Gelman and Rubin, 1992; Brooks and Gelman, 1998) and models converged well, with average Gelman's diagnostic $\hat{R} = 1.00$ for fixed and random effects, comparing chains from different priors.

3. Results

Laying date varies within a female's lifetime in response to both temperature and age (table 1). Laying date is earlier with warmer springs, with a median laying date c. 3 days earlier every 1°C warmer (table 1, figure 1). It also varies across female lifetime, getting earlier until females reached c. 4 years old, and then increasing in older ages (table 1, figure 1). Our results suggest that shorter-lived females tend to breed later, but note that the posterior distribution of the ALR 95% credible interval marginally includes zero (table 1).

We also found age-dependent plasticity in reproductive phenology. The response of laying date to temperature changes with age, as evidenced by the significant interaction between temperature and the linear age effect (table 1). This results in reaction norm slopes getting steeper as female age increases, until c. 5 years old (figure 1). In addition, our results suggest that plasticity declines for older females (after 6 years old, figure 1b,c); however small sample sizes for these late age classes result in large credible intervals (table 1, electronic supplementary material 1). The largest difference in reaction norm slopes is between 1 year-old (i.e. yearling) and 5 year-old females: the estimated median slope of laying date reaction norm with respect to temperature is $-3.68 \text{ day}/^\circ\text{C}$ (± 0.002 , p-value $< 2.10^{16}$) for yearlings, and $-4.94 \text{ day}/^\circ\text{C}$ (± 0.001 , p-value $< 2.10^{16}$) for 5 year-old females (figure 1b), leading to a median difference of c. 1.5 days/ $^\circ\text{C}$ between both age classes.

Table 1 Variation of laying date according to temperature and age. Results are from the mixed model 1 implemented with MCMCglmm. Posterior modes of estimates are indicated together with 95% credible intervals. Fixed effect estimates with 95% credible intervals that do not include zero are represented in bold.

Fixed effects	
Intercept (μ)	134.14 [132.39; 135.36]
Temperature (α_1)	-2.92 [-4.79; -1.62]
Age (α_2)	-3.43 [-3.87; -2.81]
Age² (α_3)	0.40 [0.33; 0.50]
Temperature : Age (α_4)	-0.73 [-1.31; -0.07]
Temperature : Age ² (α_5)	0.06 [-0.03; 0.15]
ALR (α_6)	-0.22 [-0.43; 0.02]
Random effects	
Year	13.80 [8.77; 22.49]
Ind	10.90 [9.05; 13.40]
Residuals	
Units (ϵ_{ij})	14.37 [13.14; 15.88]

Annual reproductive success also varies with female age (table 2). There is quadratic variation of annual reproductive success with age, such that young and especially old females display lower annual reproductive success than middle-age females (table 2, figure 2a). Annual reproductive success also decreases with laying date (table 2, figure 2b). There is no statistical evidence for an interaction between age and laying date different from zero according to model 2 (table 2). However in figure 2b, the relationship between laying date and reproductive success is negative for all ages except for female older than 5 years old. Hence, despite low statistical power in old age classes (electronic supplementary material 1), it seems that reproductive success in old females is low independently of their laying date.

4. Discussion

To our knowledge, our study is the first to report age-dependent phenological plasticity in the wild. Laying date plasticity with spring temperature is a common pattern observed in many avian natural populations (e.g. Dunn, 2004; Visser et al., 2009). In temperate regions, this plasticity causes among-year variation in phenology, which allows synchronization between predators (birds) and preys (e.g. caterpillars, spiders, see Van Noordwijk et al., 1995; Dunn, 2004). Laying date variation with female age is likewise a well-known pattern in free-living as well as in controlled iteroparous animal populations (e.g. Auld and Charmantier, 2011; Nussey et al., 2008). Similarly to previous investigations (Auld and Charmantier, 2011) model 1 shows that young and old females lay later than middle-age ones (table 1, figure 2). More importantly, we reveal changes in plasticity with age.

Table 2 Variation of annual reproductive success according to laying date and age. Results are from the mixed model 2 implemented with MCMCglmm. Posterior modes of estimates are represented together with 95% credible intervals. Fixed effect estimates with 95% credible intervals that do not include zero are represented in bold.

Fixed effects	
Intercept (γ)	3.84 [2.76; 5.25]
Age (β_1)	-0.37 [-0.64; 0.15]
Age² (β_2)	-0.01 [-0.03; -0.002]
LD (β_3)	-0.02 [-0.03; -0.01]
Age : LD (β_4)	0.002 [-0.0003; 0.006]
Random effects	
Year	0.08 [0.05; 0.14]
Ind	0.03 [0.03; 0.04]
Residuals	
Units (r_{ij})	0.03 [0.02; 0.04]

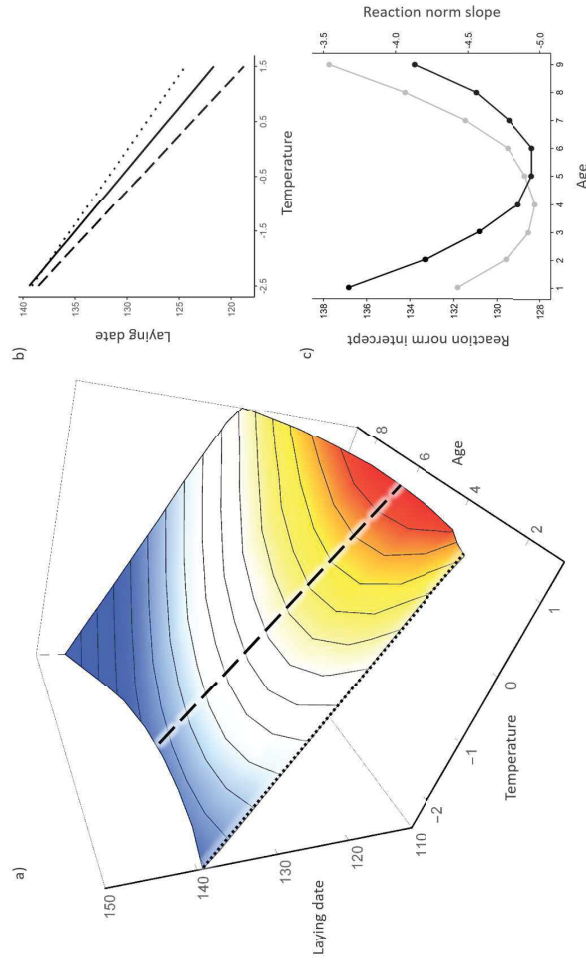


Figure 1: Age dependent reaction norm of laying date with respect to mean-centered temperature. a) Bivariate reaction norm representing predicted laying date (vertical axis and colour gradient) against temperature and age. Also represented are the univariate reaction norms with respect to the environment showing plasticity at age 1 (dotted line) and 5 (long dashed line). b) Reaction norms of predicted laying date with respect to temperature at age 1 (dotted line) and 5 (long dashed line, same as figure 1a) are shown together with the average reaction norm across all females regardless of age (solid line). For a and b, predicted values of laying date were obtained from the posterior mode of predictions across all iterations. Predictions were estimated for all combinations of temperature (from -2.5 to $+1.5$ degree around the average cue temperature) and age (from 1 to 9 years old). c) Median reaction norm intercept (in grey) and slope (in black) of laying date with respect to temperature are plotted against female age (over its observed range). The slope was estimated as $\alpha_1 + \alpha_4(A_{ij}) + \alpha_5(A_{ij}^2)$ (equation 1 and model from table 1).

The age dependence of laying date reaction norms is complex, as both the slope and intercept vary with female age (table 1, figure 1). This leads to a pattern whereby young and old females lay later on average (i.e. higher median reaction norm intercept), and the age-dependent reaction norm slope accentuates the phenological delay between young or old and middle-age females (figure 1). For instance, in warm springs with temperature 1.5 °C higher than average, the resulting median laying date is ~ 5 days earlier in 5-year-old than 1-year-old females (figure 1b). This difference among-age classes represents about 13% of the total phenotypic variation in laying date in the entire dataset (c. 39 days), while the among-individual (averaged across years and environments) and among-year variation explain respectively about 28% (c. 11 days) and about 36% (c. 14 days) of this variation.

Females of 5 years and older represent only 8% of the total population (electronic supplementary material 1 and annual adult survival probability of c. 0.5 in the population, Dubuc-Messier et al., 2016). Hence, age-dependent laying date plasticity will not necessarily have extensive impacts on population dynamics and persistence in our short-lived passerine population, under current environmental conditions. However, the effect of age-dependent plasticity on laying date among-individual variation should increase with higher spring temperature (assuming no change of laying date reaction norm through time), inducing higher among-individual variation of laying date in the future.

Our results seem consistent with theoretical models of age-dependent plasticity (e.g. Fawcett and Frankenhuis, 2015; Stamps and Krishnan, 2017; Fischer et al., 2014) despite substantial differences between our blue tit biological model and some assumptions of the theoretical models (see Introduction). As predicted by theoretical studies, plasticity decreased at old ages in our study population. However, the pattern was complex, as we observed quadratic variation of both laying date plasticity (table 1, figure 1) and annual reproductive success (table 2, figure 2). From a more mechanistic perspective, Fawcett and Frankenhuis (2015) highlight that age-dependent plasticity will evolve if there is variation across lifetime of either: i) cue reliability, ii) relationship between fitness and plastic response, and/or iii) constraints on the expression of phenotypic plasticity. First, we have no a priori reason to expect changes in cue reliability with female blue tit age: spring temperature should predict the caterpillar peak date independently of female age. However, the physiological ability to detect environmental cues such as temperature (Visser et al., 2009; Caro et al., 2013) or photoperiod (Dawson, 2008) may change with age. This could explain age-dependent plasticity since the optimal reaction norm slope increases as a function of cue reliability (Gavrilets and Scheiner, 1993), but little is known about changes in perception abilities with age in birds. Second, the fitness benefits associated with laying date plasticity may vary during female lifetime. For example, their foraging capacity may increase with age, leading to a reduced importance – for annual reproductive success – of the synchronisation between the peak of nutritive resources and bird reproductive timing in older females. In our population, such age-dependent fitness benefits variation could not explain the increasing plasticity before 5 years old.

However, the fitness advantage of earlier breeding seems to decrease in old ages (figure 2b), which could lead to lower fitness benefits of being plastic and induce selection for reduced (assumed costly) plastic capacity in old female birds. Third, a learning process of plasticity early in life may explain variation in laying date plasticity. Grieco et al. (2002) showed that blue tits lay in the same period than their previous breeding season if they were supplied in additional food, but they lay earlier if they were short of nutritive resources. Such pattern may explain increasing plasticity in young females, especially following warmer springs, when the caterpillar peak is earlier than average. Finally, plasticity is expected to require resources, especially to acquire information to track environmental changes (Dewitt, 1998). Hence, yearlings may not have accumulated enough resources during their first winter to adequately modulate their laying date according temperature, again especially in warmer years when breeding phenology is optimally early. In the same vein, old females, suffering from reproductive senescence may not be able to display steeper plasticity because of lack of resources. Further investigations about physiological conditions of young and old females are needed to better understand proximal causes of age-dependent plasticity.

While we have shown that breeding too late decreases fitness for young and middle-age but not for oldest birds, we have yet to investigate whether selection for plasticity changes with age in this population. This can be studied by analyzing how selection on laying date across environments causes indirect selection on its plasticity (e.g. van Tienderen and Koelewijn, 1994; Ramakers et al., 2018). Extending this approach to age-dependent plasticity is probably challenging, as it requires very large data sets, but is a promising development for future work.

5. Conclusion and perspectives

Considering the importance of plastic responses for population persistence (Chevin et al., 2013; Reed et al., 2010), within-individual variability of plasticity has to be taken into account in studies of plasticity in the wild. Models of age-structured populations could help to apprehend how age-related plasticity impacts adaptation and population persistence in a changing world. As chronic stress is known to speed up senescence (Ricklefs, 2008; Monaghan et al., 2008; Hayward et al., 2009), current global change could increase variation of plasticity with age, and its impact could be even stronger for phenological traits, as global change may affect the ability of organisms to display plasticity (Bonamour et al., 2019). We suggest that age-specific variation of phenological plasticity should be studied in other systems, and particularly in long-lived species, to more fully apprehend the importance of such within-individual variation of plastic capacity for population persistence in nature.

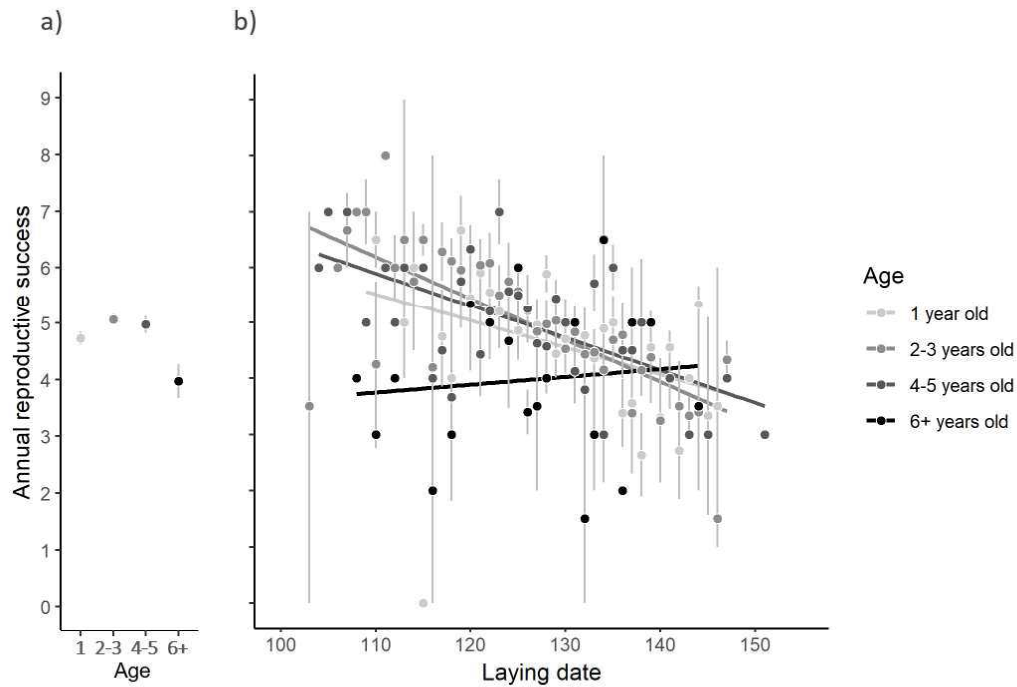


Figure 2: Influence of age and phenology on annual reproductive success. The average annual reproductive success (\pm SE) is plotted according to a) age categories, or to b) laying date and female age categories. In b), the slopes of average annual reproductive success with respect to laying date are significantly negative for the three first age classes (slope= -0.05 [SE= 0.02, p-value= 0.01], -0.08 [SE= 0.01, p-value= 4.88e-08], and -0.06 [SE= 0.01, p-value= 1.99e-05], for 1-year-old, 2-3 year-old, and 4-5 year-old females, respectively), but not for the oldest females of 6 and more years old (slope= 0.01 [SE= 0.03, p-value= 0.67]). Annual reproductive success is estimated as the number of fledglings per breeding attempt in the year. Note that some points have no standard error bars because of a single observation in the laying date – age class combination.

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Ethics

All experimental protocols described here were approved by the ethics committee for animal experimentation of Languedoc Roussillon (305-CEEA-LR-12066 approved in 2012) as well as by Regional Institutions (bylaw issued by the Prefecture on 15/06/2012 n° 2012167-0003). Captures were performed under a personal ringing permit delivered by the CRBPO (Centre de Recherches par le Bagueage des Populations d'Oiseaux) to Anne Charmantier (ringing permit number 1907).

Data accessibility

Data are available in HAL repository (hal.archives-ouvertes.fr).

Author's contributions

C.T., A.C., L.-M.C. designed the research. A.C., C.T. and S.B. (and many other contributors) collected field data. S.B. conducted statistical analyses. S.B., C.T., L.-M.C., A.C., D.R. wrote the paper.

Competing interests

We declare we have no competing interests.

Acknowledgments

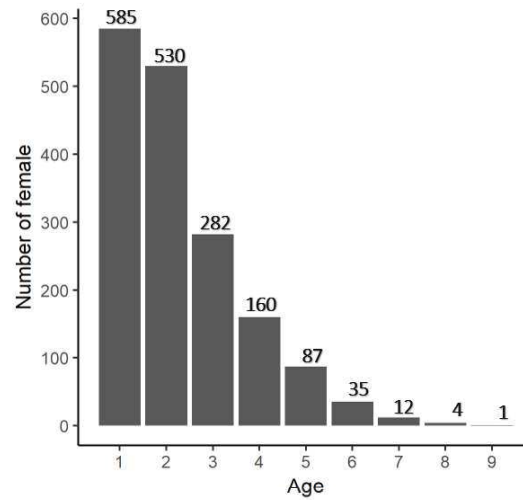
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Supplementary materials

Figure S1: Age distribution of breeding females.



Age distribution of breeding females (number of laying date observations per age classe).

Table S1: Number of laying date observations per female.

Nb. of laying date observations	1	2	3	4	5	6	7	8	total
Nb. of identified females	425	208	101	72	33	6	6	3	854



D-Muro station (Corsica).

(Credits: Anne Charmantier)

MANUSCRIPT C

Senescence in wild blue tits: Does environmental age matter?

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Manuscript in preparation

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Abstract

Senescence (or ageing), that is, performance deterioration with age, is ubiquitous and highly variable among species, populations and individuals in nature. It is unclear how environmental conditions can affect senescence trajectories within a population, and whether chronological age alone is sufficient to describe the ageing process in the wild. Here we explore the effect of environmental quality on reproductive and laying date senescence in a wild blue tit population. We disentangle the effects of chronological age and environmental quality by introducing two complementary estimations of environmental age, based on annual population-level demographic variables: the cumulative average breeding success (number of fledgling per brood) and the cumulative adult survival probability. Results show that early in life, females increase their annual reproductive success and lay earlier until they reach 3 years old, and then suffer from reproductive senescence (decreasing annual reproductive success and later laying date). These senescence patterns are influenced by both proxies of environmental age. Females experiencing better environmental conditions in their past display stronger reproductive senescence and lay later with age than females experiencing poorer environments. These results are in agreement with the disposable soma and the ‘live-fast-die-young’ hypotheses, highlighting the importance of environmental quality and variability in shaping senescence trajectories in wild birds.

Key words: Laying date, Chronological age, Environmental age, Blue tits, Senescence, Random regression model, Wild bird

1. Introduction

Senescence is characterized by increasing mortality rate (i.e. actuarial senescence) and/or decreasing annual reproductive success with age (i.e. reproductive senescence) (Rose, 1991). It is a widespread phenomenon in nature (Jones et al., 2008, 2013a; Nussey et al., 2013), and is ultimately explained by the decreasing strength of natural selection with age as the number of individuals in cohorts decline with age due to extrinsic mortality (i.e. caused by environmental factors, such as predation, parasitism or natural disaster). As a consequence, deleterious mutations expressed in old ages are not counter-selected (Fisher, 1930; Haldane, 1941; Medawar, 1946; Williams, 1957; Hamilton, 1966). There is empirical evidence for senescence variability at all living scales: i) among-species (e.g. Jones et al., 2013a,b; Peron et al., 2010; Garcia et al., 2011; Nussey et al., 2011; Schaible et al., 2015; Froy et al., 2017); ii) among-populations (e.g. Austad, 1993; Holand et al., 2016); iii) between-sexes (e.g. Hämäläinen et al., 2015; Froy et al., 2017; Murgatroyd et al., 2018); and iv) among-individuals (e.g. Descamps et al., 2009; Bouwhuis et al., 2010; Kroeger et al., 2018). Senescence has also been shown to vary among genotypes, i.e. to be heritable (e.g. Brommer et al., 2007; Wilson et al., 2007). This prompts the question: Can we explain why senescence trajectories vary at all these biological levels?

Environmental conditions encompass both ultimate and proximate causes of the diversity of senescence patterns found in nature (Lemaître and Gaillard, 2017). In terms of ultimate factors, the environment shapes senescence trajectories at the among –species and –populations scales, via its effects on extrinsic mortality and the resulting weakening of selective pressures at older ages. At the proximate level, differences in senescence trajectories among populations and individuals are the result of contemporary environmental determinants of performances such as early-life conditions (Nussey et al., 2007; Balbontin and Møller, 2015; Mumby et al., 2015; Marshall et al., 2017 and see Cooper and Kruuk, 2018 for a meta-analysis), or punctual events during adulthood, such as stress events (Shanley and Kirkwood, 2000; Roach, 2012; Hämäläinen et al., 2015). There is overall no general pattern emerging regarding how environmental quality affects senescence trajectories, and several theories have been proposed and supported in wild populations.

First, the ‘silver-spoon’ hypothesis predicts reduced senescence for individuals benefitting from the favourable long-term effects of a good early-life environment. Based on a meta-analysis of 14 wild birds and mammals studies, Cooper and Kruuk (2018) recently found broad but weak support for the ‘silver-spoon’ hypothesis for reproductive senescence, but no environmental effect on actuarial senescence. Second, the ‘live-fast-die-young’ hypothesis predicts accelerated senescence for individuals benefitting from good environments during their lifetime because of a resource allocation trade-off between survival and reproduction (e.g. Oro et al., 2014; Hooper et al., 2017; Kroeger et al., 2018). Overall, environmental effects on actuarial senescence have been studied in several species, but we know less about environmental-dependence

of reproductive senescence. Studying the impact of lifetime environmental quality on aging patterns may permit to better understand the variability of senescence trajectories observed in the tree of life.

A promising yet under-used approach for exploring environmental effects on senescence is to compile the cumulative effect of environmental stress via the concept of ‘environmental age’ (e.g. Hayward et al., 2009). Environmental age is an integrative parameter of the environmental quality experienced by a specific individual in a population. Assuming that stressful environmental conditions are linked to the accumulation of oxidative stressors, which was shown to largely drive senescence (Selman et al., 2012; Speakman et al., 2015; de Magalhães and Passos, 2018), environmental age is likely to explain senescence trajectories better than chronological age. Environmental age may be particularly useful for the study of environmental effects on senescence in wild populations, where environmental variability is uncontrolled, as opposed to laboratory experiments. Using environmental age to describe senescence pattern aims at disentangling within-individual phenotypic variation caused by i) chronological age (i.e. narrow-sense senescence), ii) experienced environmental conditions (i.e. age-independent variation of individual performances through lifetime) and iii) interactions between both, that is the variation in senescence trajectories under different lifetime environmental conditions. The concept of environmental age was introduced by Hayward et al. (2009), who showed that feral Soay sheep suffer from parasite resistance senescence, and that the increase in parasitic load with age is stronger in individuals having experienced more stressful environmental conditions during their lifetime. Surprisingly, we did not find other studies using such estimation of lifetime environmental quality. One of the reasons may be that studying senescence in wild populations is notoriously difficult because of the decreasing number of individuals with age due to extrinsic mortality.

We explored how the environment experienced over lifetime influences the age-specific patterns of annual reproductive success and laying date in a wild Mediterranean blue tit population. We estimated two integrative proxies of environmental quality based on population level parameters: i) average annual breeding success of females; and ii) adult survival probability. These proxies reflect environmental conditions in spring and the whole year (including winter) respectively.

2. Material and Methodes

2.1 Species and data collection

We carry out our analyses on a long-term study of a wild blue tit population (*Cyanistes caeruleus ogliastrae*, a small insectivorous and cavity-nesting passerine) on the Mediterranean island of Corsica, initiated by Jacques Blondel in 1975. Since 1976, breeding data is collected every spring in an evergreen forest (E-Pirio, 42°34’N, 08°44’E), dominated by holm oaks (*Quercus ilex*) (see reviews on the blue tit long-term study in Blondel et al., 2006; Charmantier

et al., 2016). Nest-boxes (supplied with surplus, 205 nest-boxes are installed and natural cavities are rare in this forest, Charmantier, pers. com.) are monitored weekly from March to June (Blondel et al., 2006) allowing to record laying date (1st January = 1) and annual reproductive success (ARS), estimated as the number of fledglings for a reproductive event of female in a given year. Birds breeding in nests-boxes are individually captured and marked with a unique numbered metal ring. We only included first broods in the analyses (second broods representing less than c. 1% of total clutches). Chronological age (from 1 to 9 years old; supplementary material 1 for number of females in each cohort) estimations are based on birth year for individuals born in our nest-boxes, or on plumage pattern for other individuals. Plumage pattern allows distinguishing one-year-old birds from birds of two years or older. In the focal dataset, exact age was known for c. 60% of females, and minimum age for c. 40%. In the same vein, the age at last reproduction (ALR) of each female was under-estimated, since only a minimum age is known, for potentially all individuals because of imperfect recapture rates (recapture probability of females equal to 0.86 [0.81; 0.90], Bastianelli et al., in prep).

2.2 Statistical analyses

First, we estimated senescence of annual reproductive success and laying date. We estimated age associated to earlier laying date and maximal annual reproductive success using models with linear and quadratic effects of age (supplementary materials 2 and 3, and figure 1). Female annual reproductive success starts decreasing after they reach 3 years old, and laying date after 4 years old. We thus estimated senescence on a subset of data that includes only females aged of more than 2 years old for annual reproductive success, or females aged of more than 3 years old for laying date, using a linear regression models:

$$Trait_{ij} = \mu + \alpha A_{ij} + ind_i + year_j + \epsilon_{ij} \quad (1)$$

with μ the population-level average trait (annual reproductive success or laying date), A_{ij} the mean-centered chronological age of females and α the rate of senescence, and ϵ_{ij} a residual component of variation. We added two random effects in order to take into account the non-independence of observations on the same individuals (ind_i) and during same years ($year_j$). Model 1 was implemented as a Poisson generalized mixed model for annual reproductive success, and as a Gaussian generalized mixed model for laying date. We also tested for female selective disappearance by including age at last reproduction as a fixed effect (supplementary material 4 and 5, respectively). We considered laying date as a female-specific trait, although males could influence laying date and its senescence because (unlike in females) there is no laying date repeatability in males (Auld and Charmantier, 2011). We tested for the origin of females (as fixed effect) in a preliminary analysis, because some studies show variation of laying date and senescence pattern according to the forest of origin in passerines (e.g. in great tits Bouwhuis et al., 2010). However, preliminary analyses did not show such origin effect in our population (not shown).

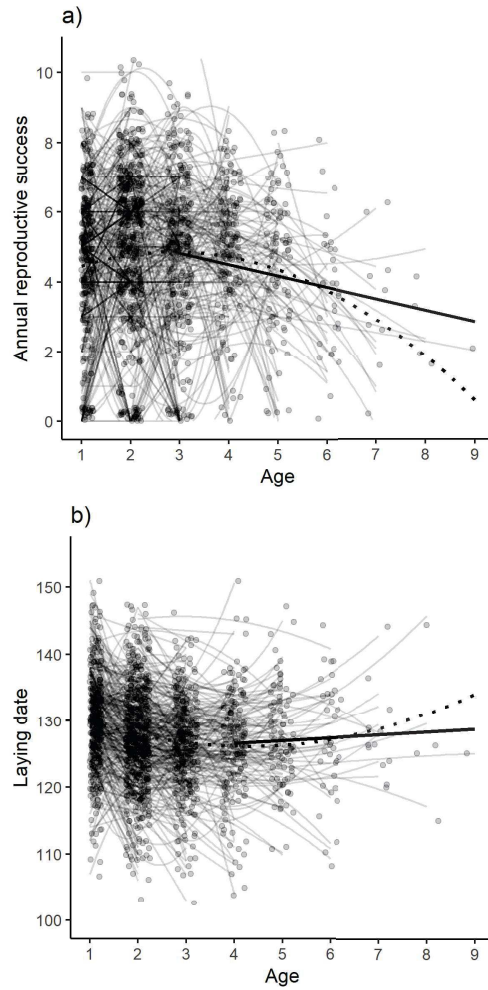


Figure 1: Reproductive senescence in blue tits. a) Annual reproductive success and b) laying date according to female chronological age. Grey lines are observed individual reaction norms. Bold black lines are population-level age-dependent trajectories, with dotted bold lines as quadratic regressions of the phenotype across ages 1 to 9 and solid bold lines linear regressions of the phenotype across ages 3 to 9 (in panel a, age vertex point with maximal annual reproductive success is c. 3, supplementary material 2) and 4 to 9 (in panel b, age vertex point with earlier laying date is c. 4, supplementary material 3).

Second, we explored environmental effects on reproductive success and laying date senescence using two integrative estimations of environmental quality. The first was cumulative fledglings number (CFN), estimated for female i in year of observation j_{obs} , as the sum of annual population average number of fledglings \bar{x}_j , from the female birth year b_i to the year before the focal year of phenotype observation (i.e. $CFN_{i,j_{obs}} = \sum_{k=b_i}^{j_{obs}-1} \bar{x}_k$). The second was cumulative adult survival (CAS), estimated for female i in year j_{obs} , as the annual adult survival probability \bar{s}_j summed for each female from her birth year to the year before the year of observation (i.e. $CAS_{i,j_{obs}} = \sum_{k=b_i}^{j_{obs}-1} \bar{s}_k$). The survival probability was estimated using capture-mark-recapture analyses (Bastianelli et al, submitted).

These two proxies for environmental quality should capture complementary facets of environmental conditions, relating to different environmental components in the life of blue tits. CFN includes survival of chicks from hatching to fledging from the nest, which depends on the local spring environment close to the nest. The number of fledglings in a brood is known to be under strong influence from the average spring temperature (Marrot et al., 2018), as well as extremely hot temperature events (Marrot et al., 2017). In addition, chicks are mainly fed with caterpillars, and the synchronization between bird and prey phenologies are crucial for reproductive success. Years with synchronized bird reproductive period and caterpillar peak date of abundance are expected to be associated with higher average number of fledglings (e.g. Visser et al., 2006). Thus, female with high values of CFN are on average females experiencing less hot years, without extreme climatic events and a good ‘bird – prey’ synchronisation. In contrast, CAS is expected to be more closely related to the environment experienced by adults during the entire year in the forest, especially the winter season where highly adverse conditions such as frosting may occur (Grosbois et al., 2006). Although the effort to feed the chicks during the breeding season is higher in years with poor synchronisation between bird and prey phenologies, we expected adult survival to be less influenced by caterpillar phenologies than the number of fledglings. In summary, females with high values of CFN and CAS experienced more favourable environmental conditions in their past. Note however that a higher adult survival probability and/or fledgling number in a given year can be associated with higher population density during the following year, which may lead to lower adult survival and number of fledglings due to density dependence.

We implemented models with environmental age in two ways, including it either: i) as a continuous fixed variable, whereby both CFN and CAS are positively correlated with chronological age (supplementary material 6), or ii) as a categorical variable not correlated with chronological age, where both CFN and CAS were mean-centered and divided in three categories within each chronological age (‘bad’: lower 33% values i.e. worse environmental quality, ‘medium’: median 33% values i.e. median environmental quality, ‘good’: higher 33% values i.e. best environmental quality). We estimated the effects of environmental age on annual reproductive success and laying date variation using the following models:

$$Trait_{ij} = \mu + \alpha_a A_{ij} + \alpha_b E_{ij} + \alpha_b(A_{ij}E_{ij}) + ind_i + year_j + \epsilon_{ij} \quad (2)$$

where $Trait_{ij}$ (annual reproductive success or laying date) is explained by mean-centered chronological age A_{ij} (females of 3 years and older for annual reproductive success or females of 4 years and older for laying date). Environmental age E_{ij} (CFN or CAS) and its interaction with chronological age ($A_{ij}E_{ij}$), which estimates the effect of lifetime environmental quality on average trait and senescence patterns. As for previous models, we included female identity (ind_i) and year ($year_j$) as random effects.

All statistical analyses were carried out using the R software (version 3.4.4, R Core Team, 2018) and performed in a Bayesian framework using a Markov

Chain Monte Carlo estimation algorithm (R package MCMCglmm, Hadfield, 2010). For random effects and residual variance we used low informative priors corresponding to an inverse-Wishart $w^{-1}(\psi, \nu)$ distribution, with $\nu = 1$ and ψ equal to the phenotypic variance of laying date or annual reproductive success (depending on the model) divided by the number of estimated variance components. The default Gaussian non-informative prior in MCMCglmm with mean zero and variance 10^8 was used for the fixed effects. Each model ran for 1,010,000 iterations, saved each 1000 iteration to avoid autocorrelation, and using a burn-in period of 10,000 iterations. Maximum autocorrelation between estimates was < 0.05 for fixed and random effects.

3. Results

3.1 Reproductive and laying date senescence

Annual reproductive success varies along female lifetime, with an increase until c. 3 years old followed by a decrease in old females (figure 1a, supplementary material 2). The decreasing pattern of annual reproductive success is confirmed by the linear model fitted on females of three years and older (model 1, table 1a and figure 1a). Females living longer have a higher breeding success than shorter lived females (supplementary material 4), suggesting selective disappearance of females with lower annual reproductive success (model run on females of 1 to 9 years old). However, this effect is not detected if the model only includes females older than 2 years (supplementary material 2). Hence, we did not include an effect of age at last reproduction in subsequent models.

Laying date also varies with female age and age-squared (figure 1b, supplementary material 3) with progressively earlier laying in the beginning of a female's reproductive life followed by increasingly delayed laying with age after c. 4 years old. The delayed laying in older females is confirmed in a model run on females of 3 years and older (model 1, table 1b, figure 1b). There is no evidence for an effect of age at last reproduction on laying date (supplementary material 5), and thus no selective disappearance according to a female's average laying date.

Altogether, results show that there is reproductive senescence in this blue tit population for both annual breeding success and laying date.

3.2 Reproductive performances across age and environmental quality

Both average annual fledging number and adult survival probability show substantial annual variation, with annual average number of fledglings ranging from 1 to 6 (figure 2a) and adult survival probability from 0.31 to 0.7 (figure 2b). These two estimates of annual environmental quality are not correlated (slope: -0.02 ± 0.02 , p-value = 0.17, $r^2 = 0.03$), suggesting that they represent two complementary and independent facets of average environmental conditions.

Table 1 Senescence in blue tit. a) Annual reproductive success as a function of female chronological age (model implemented as a generalized mixed model with Poisson distribution); b) Laying date as a function of chronological age. We present posterior modes and their 95% credible interval. Chronological age is mean-centered and the data set only includes females of 3 years and older for annual reproductive success and females of 4 years and older for laying date. In bold, fixed effects estimates with credible interval excluding zero.

a) Annual reproductive success	
Fixed effects	
Intercept	1.45 [1.14; 1.74]
Age	-0.08 [-0.14; -0.02]
Random effects	
Year	0.64 [0.40; 1.02]
Ind	0.25 [0.20; 0.29]
Residuals	
Units	0.23 [0.19; 0.29]

b) Laying date	
Fixed effects	
Intercept	126.36 [125.02; 129.11]
Age	0.28 [0.02; 0.63]
Random effects	
Year	30.11 [19.38; 53.35]
Ind	9.87 [7.18; 13.16]
Residuals	
Units	10.41 [8.66; 12.35]

Both estimates of environmental age (cumulative fledglings number, CFN, and cumulative adult survival, CAS) influence the senescence rate of annual reproductive success (table 2). When environmental age is implemented as a continuous variable, we find negative interactions between chronological (females aged of 3 years and older) and environmental age (table 2a,b). This means that the decrease in reproductive performance with age is more marked for higher values of the CFN or CAS, indicative of faster senescence. When environmental age is implemented as a categorical variable, average annual reproductive success during the entire lifetime is higher for females benefitting from higher environmental quality (for instance females in CFN categories ‘medium’ and ‘good’ had on average c. 0.5 fledglings more than females in the worst environmental quality ‘bad’, figure 3a). Although figures 3c,d suggest that the decline in annual reproductive success in old ages is stronger in better environmental conditions both for CFN and CAS, the difference is not detectable for CFN, and the interaction between age and CAS_{good} is negative but its credible interval marginally include zero (table 2d), probably because of low statistical power (see supplementary material 1 for sample sizes). Altogether, despite low statistical power when environmental age is implemented as categorical variables, results suggest that females benefitting from good envi-

ronmental conditions show a steeper improvement of their reproductive performance in their first breeding years, yet display stronger reproductive senescence in old ages (figure 3), to reach approximatively the same value of annual reproductive success than females experiencing poorer environment throughout their life.

Laying date varies with both CFN and CAS (model 2, table 3). Models including environmental age as a continuous variable show that females lay on average earlier when they benefited from good environmental conditions in the past (table 3a,b, note that the CAS credible interval includes marginally zero, but its posterior mode suggests a strong negative effect, table 3b). In addition to environmental effects on average laying date, both CFN and CAS interact positively with chronological age. These results suggest that females experiencing favourable environments laid on average earlier yet display stronger senescence (later breeding) in their later reproductive years. When environmental age is implemented as a categorical variable, models show similar qualitative results (figure 3g-h, table 3c,d).

Altogether, these results reveal that young and middle age females experiencing higher environmental quality perform better than females experiencing poorer environments, but display stronger reproductive and laying date senescence (figure 3).

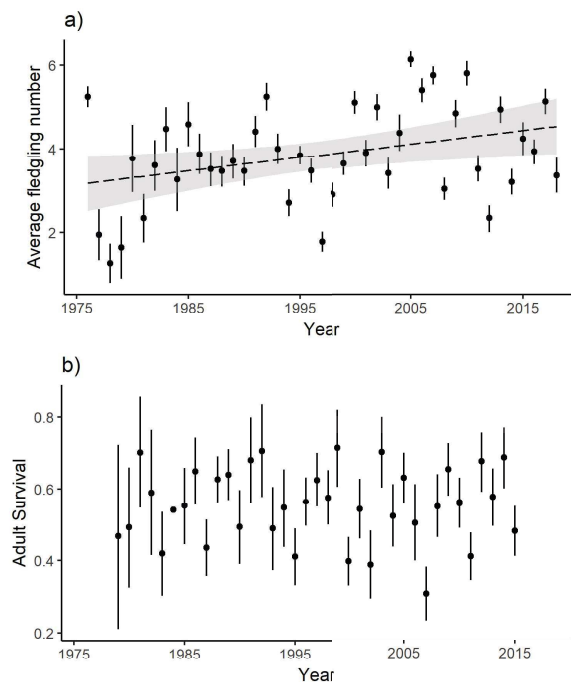


Figure 2: Temporal fluctuations in average annual reproductive success and adult survival probability. a) Annual variation in the average number of fledglings per brood (\pm standard errors) (slight increase with time: slope \pm SE = 3.85 ± 1.58 , p-value = 0.02, $r^2 = 0.10$; however note that the correlation is no robust when the first 4 years are removed from the data sets; b) Annual variation in adult survival probability (\pm standard errors) (no trend in adult survival probability with time: slope \pm SE = -0.28 ± 17.48 , p-value = 0.99, $r^2 = 0.03$).

Table 2 Reproductive senescence and environmental age. Annual reproductive success as a function of chronological age and environmental age. In a) cumulative fledgling number (CFN) and b) cumulative adult survival (CAS), environmental age is implemented as a continuous variable. In c) and d) panels CFN and CAS are mean-centered and divided in three categories within each chronological age ('bad': lower 33%, 'medium': median 33%, 'good': higher 33%, with the 'bad' category as reference level). We present posterior modes and their 95% credible interval. Chronological age is mean-centered and the data set only includes females of 3 years and older. All models are implemented as a generalized linear mixed model with Poisson distribution. In bold, fixed effect estimates with 95% credible intervals not including zero. In italic, fixed effect estimates marginally excluding in the 95% credible interval.

a) CFN		b) CAS	
	Fixed effects		Fixed effects
Intercept	0.84 [-0.05, 1.55]	Intercept	1.17 [-0.14, 1.97]
Age	0.02 [-0.33, 0.30]	Age	0.16 [-0.31, 0.63]
CFN	0.05 [-0.02, 0.14]	CAS	0.12 [-0.50, 1.10]
Age : CFN	-0.01 [-0.02, -0.002]	Age : CAS	-0.11 [-0.18, -0.03]
	Random effects		Random effects
Year	0.61 [0.41, 1.00]	Year	0.70 [0.40, 1.08]
Ind	0.23 [0.20, 0.31]	Ind	0.26 [0.21, 0.33]
	Residuals		Residuals
Units	0.23 [0.19, 0.28]	Units	0.25 [0.19, 0.29]
c) CFN		d) CAS	
	Fixed effects		Fixed effects
Intercept	1.28 [-0.89, 1.68]	Intercept	1.42 [0.93, 1.76]
Age	-0.02 [-0.15, 0.09]	Age	-0.05 [-0.16, 0.08]
CFN _{medium}	0.82 [-0.12, 0.71]	CAS _{medium}	-0.08 [-0.46, 0.52]
CFN _{good}	0.98 [-0.19, 0.77]	CAS _{good}	0.25 [-0.18, 0.74]
Age : CFN _{medium}	-0.22 [-0.26, 0.11]	Age : CAS _{medium}	0.007 [-0.18, 0.19]
Age : CFN _{good}	-0.27 [-0.23, 0.12]	Age : CAS _{good}	<i>-0.14 [-0.30, 0.05]</i>
	Random effects		Random effects
Year	0.69 [0.40, 1.04]	Year	0.67 [0.42, 1.12]
Ind	0.24 [0.20, 0.32]	Ind	0.26 [0.20, 0.33]
	Residuals		Residuals
Units	0.23 [0.19, 0.29]	Units	0.23 [0.19, 0.30]

Table 3 Laying date senescence and environmental age. Laying date as a function of chronological age and environmental age. In a) cumulative fledgling number (CFN) and b) cumulative adult survival (CAS), environmental age is implemented as a continuous variable. In c) and d) both environmental age estimates CFN and CAS are mean-centered and divided in three categories within each chronological age ('bad': lower 33%, 'medium': median 33%, 'good': higher 33%, with the 'bad' category as reference level). We present posterior modes and their 95% credible interval. Chronological age is mean-centered and the data set only includes females of 4 years and older. In bold, fixed effect estimates with 95% credible intervals no including zero. In italic, fixed effect estimates marginally including zero in the 95% credible interval.

a) CFN		b) CAS	
Fixed effects		Fixed effects	
Intercept	132.68 [128.65, 137.40]	Intercept	132.79 [127.79, 138.87]
Age	0.99 [-0.27, 3.06]	Age	1.08 [-1.92, 2.85]
CFN	-0.51 [-1.06, -0.22]	CAS	-2.91 [-7.70, 0.50]
Age : CFN	0.04 [0.01, 0.09]	Age : CAS	0.42 [0.17, 0.77]
Random effects		Random effects	
Year	27.61 [17.73, 49.00]	Year	24.32 [14.51, 40.1]
Ind	9.30 [7.21, 12.96]	Ind	9.76 [7.18, 12.83]
Residuals		Residuals	
Units	9.61 [8.33, 11.81]	Units	9.70 [8.38, 11.87]
c) CFN		d) CAS	
Fixed effects		Fixed effects	
Intercept	128.73 [124.19, 130.92]	Intercept	128.89 [125.31, 132.06]
Age	0.32 [-0.52, 1.00]	Age	0.04 [-0.87, 0.91]
CFN _{medium}	-6.40 [-9.38, -1.77]	CAS _{medium}	-1.40 [-6.93, 1.55]
CFN _{good}	-3.64 [-7.77, 1.23]	CAS _{good}	-6.74 [-11.53, -2.53]
Age : CFN _{medium}	1.46 [0.16, 2.47]	Age : CAS _{medium}	0.49 [-1.13, 1.98]
Age : CFN _{good}	0.34 [-0.78, 1.47]	Age : CAS _{good}	1.65 [0.27, 2.66]
Random effects		Random effects	
Year	31.28 [22.07, 63.22]	Year	26.27 [16.52, 51.68]
Ind	8.51 [6.16, 14.30]	Ind	8.89 [6.02, 13.73]
Residuals		Residuals	
Units	8.88 [7.16, 12.25]	Units	8.96 [6.93, 11.83]

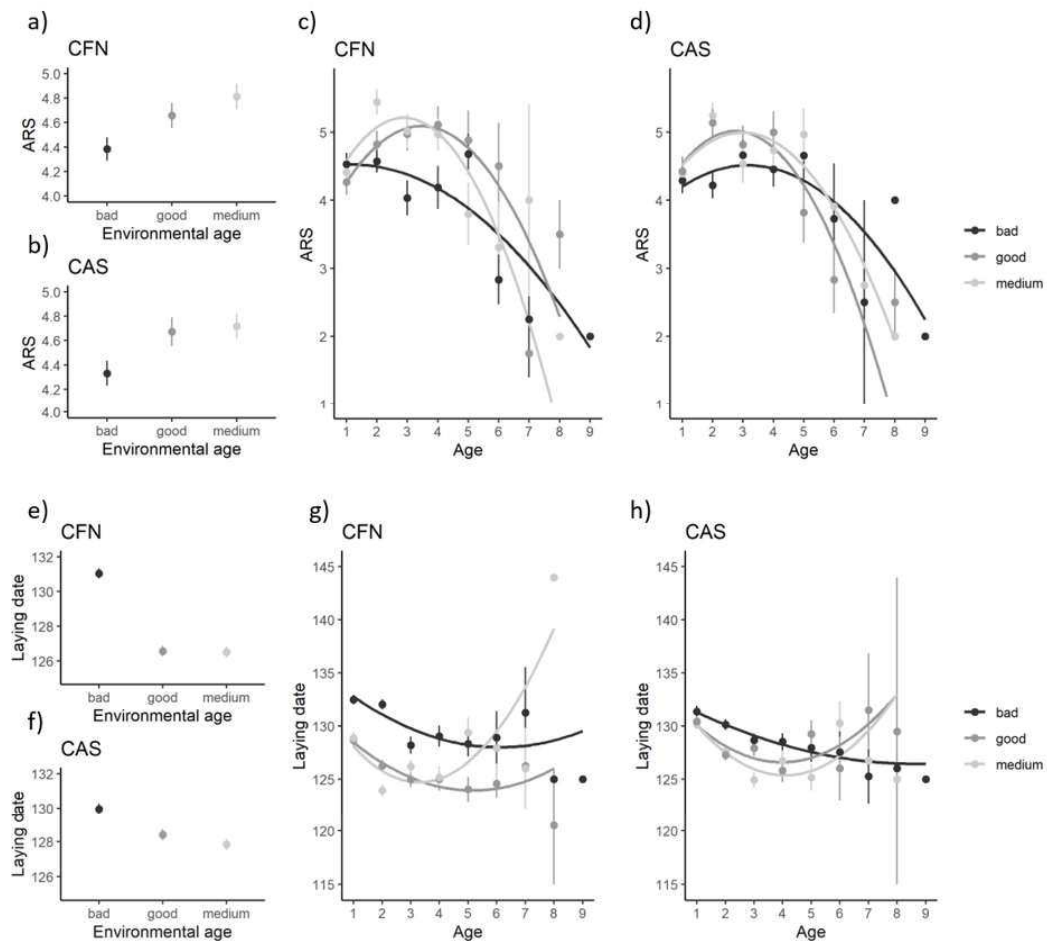


Figure 3: Senescence trajectories vary with environmental age. (a and b) Average annual reproductive success (\pm standard error) according to environmental age for a) cumulative fledgling number (CFN) and b) cumulative adult survival (CAS). (c and d) Average annual reproductive success (\pm standard error) variation according to chronological and environmental age c) CFN and d) CAS. (e and f) Average laying date (\pm standard error) according to environmental age estimated by e) CFN and f) CAS; (g and h) Average laying date (\pm standard error) in relation with chronological age and environmental age g) CFN and h) CAS). Both environmental age estimates CFN and CAS are mean-centered and divided in three categories within each chronological age ('bad': lower 33% i.e. worse environmental quality, 'medium': median 33% i.e. median environmental quality, 'good': higher 33% i.e. best environmental quality). Chronological age is ranging from 1 to 9 years old.

4. Discussion

Using individual data from a long-term study of a wild blue tit population, we show that female reproductive performances vary with age, and that old females display both reproductive and laying date senescence (as previously shown in this blue tit population, Auld and Charmantier, 2011, and in the great tit sister species, Bouwhuis et al., 2009). Senescence trajectories are however dependent on experienced environmental conditions (table 2 and 3,

figure 3). Using two integrative estimations of environmental quality – the cumulative fledgling number ‘CFN’ and the cumulative adult survival ‘CAS’ – we show that females experiencing more favourable environments in their past have on average higher reproductive performances than females experiencing unfavourable environments, yet they present stronger reproductive and laying date senescence (figure 3). Comparing with other wild populations is to this day difficult because to our knowledge, our study is the first to investigate how patterns of avian reproductive and phenological senescence are affected by lifetime environmental quality. As Hayward et al. (2009) working on Soay sheep, we find an influence of environmental age on senescence trajectories, which suggests that the cumulative lifetime environmental quality may allow a better description and understanding of senescence patterns in natural populations as compared to considering only chronological age.

Our results corroborate the ‘live-fast-die-young’ hypothesis (e.g. Robinson et al., 2006) since females experiencing favourable environments lay earlier and have more broods when young and middle aged, yet display stronger senescence when old. The ‘live-fast-die-young’ hypothesis is in accordance with the disposable soma hypothesis, stating that limitation in resources allocated to both body maintenance and reproduction leads to resource allocation trade-offs, such that high reproductive success in early life should be associated to stronger senescence (Kirkwood, 1977; Kirkwood and Holliday, 1979).

We restricted our analyses to female-specific variation in reproductive performances, but senescence trajectories and stress resistance could be different between the two sexes (Hämäläinen et al., 2015; Hayward et al., 2015; Dantzer and Garratt, 2017; Watson et al., 2017) and there can be interactions between sex-specific life-history trajectories (Auld and Charmantier, 2011). An extended research perspective would to study sex differences in environmental effect on senescence trajectories.

Laying date age-dependent patterns varied with environmental quality, when estimated by cumulative environmental age. In a temporally variable environment, individuals of different cohorts will experience different environmental ages, resulting from different average laying dates, reproductive successes and age-specific trajectories of reproductive performances. Consequently, a variable environment will be associated with larger among-individual phenotypic variability of laying date, of its age-specific pattern and of its reproductive senescence. Blue tits are known to be sensitive to extreme temperature events for phenological plasticity (Bonamour et al., 2019) and reproductive success (Marrot et al., 2017). In a context of global change, causing increased frequency and intensity of extreme climatic events (ECE) (as observed in our study site) we thus may expect an increase in the variability of reproductive performances and senescence patterns in the focal population in the future.

Environmental age as estimated by average fledgling number and adult survival probability does not provide knowledge on the exact environmental driver(s) of variation in age-dependent reproductive success and laying date. These variables however represent meaningful integrative estimations of environmental quality, which is inherently highly multivariate and variable. Exper-

imental studies will be necessary to identify such causal environmental drivers in the future. Previous studies revealed that senescence trajectories seem to be at least partly driven by oxidative stress accumulation, either caused by stressful environmental conditions (e.g. Hämäläinen et al., 2015; Speakman et al., 2015) or by internal stress related for example to high reproductive effort. Since senescent individuals are less resilient to stress, environmental effects on senescence can be stronger for very old individuals, thereby accelerating the senescence in a positive feedback loop. The study of molecular and physiological processes at the origin of environmental effects of stressors on senescence trajectories will be a fruitful future research area.

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Ethics

All experimental protocols described here were approved by the ethics committee for animal experimentation of Languedoc Roussillon (305-CEEA-LR-12066 approved in 2012) as well as by Regional Institutions (bylaw issued by the Prefecture of Haute-Corse on 17/04/2018 n° 2B-2018-04-17-001). Captures were performed under a personal ringing permit delivered by the CRBPO (Centre de Recherches par le Bagueage des Populations d'Oiseaux) to Anne Charmantier (ringing permit number 1907).

Data accessibility

Data are available in HAL repository (hal.archives-ouvertes.fr).

Author's contributions

S.B., C.T., A.C. and L.-M.C. designed the research. A.C., C.T., O.B. and S.B. (and many other contributors) collected field data. S.B. conducted the analyses. O.B. provided annual adult survival. S.B., O.B., C.T., L.-M.C. and A.C. wrote the paper.

Competing interests

We declare we have no competing interests.

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Supplementary materials

Supplementary material 1

Sample sizes for senescence analyses (both annual reproductive success and laying date, model 1 in the main text and models S2, S3, S4 and S5 in supplementary materials) and for model with cumulative fledgling number (CFN) as environmental age (model 2 in the main text). Analyses were run on a dataset collected between 1979 and 2017 (39 years).

Age	1	2	3	4	5	6	7	8	9	Σ
Number of obs.	585	530	282	160	87	35	12	4	1	1696

Number of obs.	1	2	3	4	5	6	7	8	Σ
Number of identified females	422	208	101	72	33	6	6	3	851

Sample sizes for models with cumulative adult survival (CAS) as environmental age (model 2 in the main text). Analyses were run on a dataset collected between 1980 and 2015 (36 years).

Age	1	2	3	4	5	6	7	8	9	Σ
Number of laying date obs.	536	487	262	154	85	34	12	4	1	1575

Number of obs.	1	2	3	4	5	6	7	8	Σ
Number of females	387	186	93	70	31	6	6	3	782

Supplementary material 2

Age-specific variation in annual reproductive success.

We estimated variation in annual reproductive success over female lifetime (1 to 9 years old) as:

$$ARS_{ij} = \mu + \alpha_1 A_{ij} + \alpha_2 A_{ij}^2 + ind_i + year_j + \epsilon_{ij} \quad (S2)$$

with annual reproductive success ARS of a female i during the year j described by the average population-level annual reproductive success μ and chronological age and age-squared effects (A_{ij} and A_{ij}^2 , mean-centered). Annual reproductive success observed on the same individuals and the same years are non-independent, we thus added two random effects: female identity (ind_i) and year ($year_j$). ϵ_{ij} are residuals. Model S2 was implemented as a generalized mixed model with Poisson distribution.

Table S2: results of the model S2. We present posterior modes and their 95% credible interval. In bold, fixed effect estimates with credible interval excluding zero.

Fixed effects	
Intercept	1.38 [1.15, 1.66]
Age	0.04 [0.01, 0.08]
Age²	-0.04 [-0.05, -0.02]
Random effects	
Year	0.59 [0.42, 1.00]
Ind	0.13 [0.11, 0.16]
Residuals	
Units	0.12 [0.11, 0.15]

We used same model as equation S2 implemented as Gaussian ARS for estimation of vertex point annual reproductive success variation with age. The maximal annual reproductive success is found at 2.83 years old.

Supplementary material 3

Laying date variation with age.

We modelled laying date through female lifetime (1 to 9 years old) as:

$$LD_{ij} = \mu + \alpha_1 A_{ij} + \alpha_2 A_{ij}^2 + ind_i + year_j + \epsilon_{ij} \quad (\text{S3})$$

where the laying date LD of a female i in year j is explained by μ , the average laying date of the population, the female's chronological age and age-squared (A_{ij} and A_{ij}^2 , respectively, and chronological age was mean-centered) and two random effects: female identity ind_i and year $year_j$. ϵ_{ij} are residuals.

Table S3: Results of the model S3. We present posterior modes and their 95% credible interval. In bold, fixed effect estimates with credible interval excluding zero.

Fixed effects	
Intercept	128.28 [126.59, 130.02]
Age	-1.54 [-1.73, -1.31]
Age²	0.41 [0.34, 0.50]
Random effects	
Year	26.39 [17.29, 42.29]
Ind	11.15 [9.26, 13.55]
Residuals	
Units	14.39 [13.1, 15.74]

We calculated the vertex point for laying date variation with age using estimates from the model S4: the earlier laying date is found at 4.19 years old.

Supplementary material 4

Female selective disappearance according to their annual reproductive success.

Variation in annual reproductive success variation across female age could be confounded with selective disappearance of females according to their average phenotype (e.g. Bouwhuis et al., 2009). For instance, if longer-lived (hence longer breeding) females have lower reproductive success, the early disappearance of high performing females could be confounded with a decline in breeding performance with age. In an even more complex situation, short-lived females could have different ageing patterns from long-lived females. We tested for selective disappearance of females according to their annual reproductive success with the following model:

$$ARS_{ij} = \mu + \alpha_1 A_{ij} + \alpha_2 A_{ij}^2 + \alpha_3 ALR_i + \alpha_4 A_{ij} ALR_i + \alpha_5 A_{ij}^2 ALR_i + ind_i + ind_i A_{ij} + year_j + \epsilon_{ij} \quad (S4a)$$

where annual reproductive success ARS of female i during year j is explained by fixed effects A_{ij} and A_{ij}^2 , respectively female i age and age-squared in year j . We controlled for female longevity with ALR_i fixed effect corresponding to the age at last reproduction of female i . The $\alpha_3 A_{ij} ALR_i$ term fits an interaction between A_{ij} and ALR_i , and aimed at estimating selective disappearance of individuals according to their aging pattern. We added three random effects: female identity ind_i , the interaction between female identity and their chronological age $ind_i A_{ij}$ and finally a year effect $year_j$. The final ϵ_{ij} term is the residual error term. Chronological age ranged from 1 to 9 years old and was mean-centered in order to allow biological interpretations on senescence reactions norms.

Table S4a: Results of the model S4a. We present posterior modes and their 95% credible interval. In bold, fixed effect estimates with credible interval not including zero. In italic, fixed effect estimates marginally including zero in the 95% credible interval.

Fixed effects	
Intercept	1.28 [1.03, 1.61]
Age	0.00 [-0.14, 0.14]
Age²	-0.8 [-0.16, -0.02]
Age at last reproduction (ALR)	0.03 [0.01, 0.08]
Age : ALR	0.01 [-0.03, 0.04]
<i>Age² : ALR</i>	<i>0.01 [0.00, 0.02]</i>
Random effects	
Year(ARS)	0.55 [0.41, 1.02]
Ind(ARS)	0.14 [0.12, 0.17]
Ind(Age)	0.13 [0.11, 0.13]
Residuals	
Units	0.13 [0.11, 0.15]

We detect very slight selective disappearance of females according to their reproductive success in the data set: females with higher average reproductive success live a bit longer than females with lower reproductive success for a given age.

Model with females aged of 3 years and more:

$$ARS_{ij} = \mu + \alpha_1 A_{ij} + \alpha_2 ALR_i + \alpha_3 A_{ij} ALR_i + ind_i + ind_i A_{ij} + year_j + \epsilon_{ij} \quad (S4b)$$

Table S4b: Results of the model S4b. We present posterior modes and their 95% credible interval. In bold, fixed effect estimates with credible interval excluding zero.

Fixed effects	
Intercept	1.15 [0.39, 1.64]
Age	0.07 [-0.53, 0.55]
ALR	0.06 [-0.02, 0.20]
Age : ALR	-0.04 [-0.12, 0.08]
Random effects	
Year(ARS)	0.70 [0.42, 1.09]
Ind(ARS)	0.33 [0.25, 0.41]
Ind(Age)	0.23 [0.19, 0.30]
Residuals	
Units	0.25 [0.20, 0.33]

We detected no selective disappearance of females according to their reproductive success when only taking into account females of 3 years or older.

Sample size could be insufficient to allow enough statistical power. Overall, based on results from models S3.a and S3.b, we did not include an ALR effect in the main models because selective disappearance was either very small or inexistent.

Supplementary material 5

Female selective disappearance according to their laying date.

Laying date variation with age could be confounded with selective disappearance of females according to their average laying date (e.g. if late breeding females lived longer Bouwhuis et al., 2009). We tested for selective disappearance of female according to their laying date with the following model:

$$LD_{ij} = \mu + \alpha_1 A_{ij} + \alpha_2 ALR_i + \alpha_3 A_{ij} ALR_i + ind_i + ind_i A_{ij} + cov(ind_i, ind_i A_{ij}) + year_j + \epsilon_{ij} \quad (S5)$$

where the laying date LD of female i during year j was explained by fixed effects A_{ij} and female longevity (approximated with the age at last reproduction ALR_i). The $\alpha_3 A_{ij} ALR_i$ interaction term aimed at estimating selective disappearance of individuals according to their laying date aging pattern. Laying date observations are not independent because of multiple reproductive events per female, thus we added female identity ind_i as a random effect. We also estimated among-individual variation of aging by fitting the $ind_i A_{ij}$ random effect and the covariance between female identity and among-individual senescence ($cov(ind_i, ind_i A_{ij})$). Because of uncontrolled environmental effects on laying date (e.g. Porlier et al., 2012), we added year as a random effect $year_j$. The final term in equation S2 is the residual error term ϵ_{ij} . Age ranged from 3 to 9 years old (we removed young individuals less than 3 in order to model linear reaction norm) and was mean-centered in order to allow biological interpretations on senescence reactions norms (Morrissey and Liefing, 2016). We implemented heterogeneous residual structure (mean-centered age was divided in three equal categories of c. 33% of the total number of females) in order to estimate unbiased random effects (e.g. Nicolaus et al., 2013; Ramakers, 2019).

Table S5: Results of the model S5. We present posterior modes and their 95% credible interval. In bold, fixed effect estimates with credible interval excluding zero.

Fixed effects	
Intercept	128.97 [125.57, 131.07]
Age	-1.15 [-3.82, 0.57]
ALR	-0.35 [-0.82, 0.09]
Age : ALR	0.22 [-0.10, 0.74]
Random effects	
Year(LD)	29.76 [20.1, 53.18]
Ind(LD)	13.53 [11.60, 18.24]
Ind(Age)	6.97 [3.90, 9.97]
Cov(Ind(LD), Ind(Age))	1.96 [0.13, 4.48]
Residuals	
Units 1	4.87 [3.72, 5.47]
Units 2	2.71 [1.92, 4.01]
Units 3	4.52 [3.61, 6.29]

We detected no selective disappearance of female according to their laying date in this data set.

Supplementary material 6

(Co)variance and coefficient of determination (r^2) between both environmental ages: cumulative chick survival (CFN) and cumulative adult survival (CAS), and chronological age. The diagonal shows the chronological and environmental age variances.

	CFN	CAS	Age
CFN	$\sigma^2 = 31.68$	4.24, ($r^2 = 0.85$)	7.51, ($r^2 = 0.90$)
CAS		$\sigma^2 = 0.59$	1.07, ($r^2 = 0.85$)
Age			$\sigma^2 = 1.88$

This table shows high covariances and correlations across all age measures.



View from the fieldwork house in E-Pirio station.

(Credits: Anne Charmantier)

Chapter Three

Natural selection on a phenological
trait, the laying date

MANUSCRIPT D

Selection on skewed characters and the paradox of stasis

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This paper is the result of my master internship (of 5 months) co-supervised by Luis-Miguel Chevin, Céline Teplitsky, Anne Charmantier and Pierre-André Crochet. I finished analyses and wrote the paper during the first months of my PhD, it is thus included as part of my PhD work, although it may seem at first glance slightly outside the main scope of the thesis, i. e. phenotypic plasticity.

However, the topic of this study can be related to the whole thesis as follow. In this manuscript, we studied the effect of the shape of the phenotypic distribution of quantitative traits on the estimation of natural selection gradient. The phenotypic trait was decomposed into its genetic and environmental components. In other words, we studied the influence of the shape of phenotypic distribution on natural selection estimation according to its origin: heritable or plastic.

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Abstract

Observed phenotypic responses to selection in the wild often differ from predictions based on measurements of selection and genetic variance. An overlooked hypothesis to explain this paradox of stasis is that a skewed phenotypic distribution affects natural selection and evolution. We show through mathematical modelling that, when a trait selected for an optimum phenotype has a skewed distribution, directional selection is detected even at evolutionary equilibrium, where it causes no change in the mean phenotype. When environmental effects are skewed, Lande and Arnold's (1983) directional gradient is in the direction opposite to the skew. In contrast, skewed breeding values can displace the mean phenotype from the optimum, causing directional selection in the direction of the skew. These effects can be partitioned out using alternative selection estimates based on average derivatives of individual relative fitness, or additive genetic covariances between relative fitness and trait (Robertson-Price identity). We assess the validity of these predictions using simulations of selection estimation under moderate samples size. Ecologically relevant traits may commonly have skewed distributions, as we here exemplify with avian laying date – repeatedly described as more evolutionarily stable than expected – so this skewness should be accounted for when investigating evolutionary dynamics in the wild.

Key words: Paradox of stasis, Phenotypic skewness, Response to selection, Selection gradient, Selection estimation

1. Introduction

One of the most striking facts revealed by studies of evolutionary dynamics and natural selection on wild organisms over the past fifty years is the relative temporal stability of heritable phenotypes, despite the detection of directional selection with rather consistent direction in time (Kingsolver, 2001; Kingsolver and Pfennig, 2007; Merilä et al., 2001; Morrissey and Hadfield, 2011). Such absence of evolution of heritable traits under apparent directional selection constitutes the so-called “paradox of stasis” at the micro-evolutionary scale (the macro-evolutionary paradox of stasis involves absence of long-term change despite the possibility of rapid evolution, Estes and Arnold, 2007; Gingerich, 2009).

Several non-exclusive hypotheses have been formulated to explain this paradox (Merilä et al., 2001; Morrissey et al., 2010; Stinchcombe and Kirkpatrick, 2012; Walsh and Blows, 2009). For instance, the multivariate nature of phenotypes under selection may induce an over-estimation of heritability by non-multivariate approaches (due to genetic correlations between traits, Hansen and Houle, 2004; Teplitsky et al., 2014), or spurious estimates of selection may be caused by purely environmental covariances between phenotypes and fitness (Bonnet et al., 2017; Rausher, 1992; Morrissey et al., 2010). At present, there is no consensus as to the origin of this evolutionary paradox (Rollinson and Rowe, 2015; Kaplan, 2009; Estes and Arnold, 2007; Haller and Hendry, 2013; Hansen and Houle, 2004; Merilä et al., 2001), and while several processes are most probably involved, there is insufficient empirical evidence to conclude on their relative importance.








One phenomenon that could contribute to the paradox of stasis but has so far been largely neglected is skewness in phenotypic distributions. Quantitative genetics theory and statistical analyses mostly rest on the assumption of Gaussian distributions of phenotypes, breeding values, and environmental effects (e.g. Lynch and Walsh, 1998; Falconer and MacKay, 1996). This assumption is commonly made for mathematical convenience, but is also biologically justified when variation in a quantitative trait is determined by a large number of loci with infinitesimal effects (Falconer and MacKay, 1996; Fisher, 1918; Turelli and Barton, 1994), or by a balance between stabilizing selection and high mutation rates even at a few loci (Kimura, 1965; Lande, 1976b). However, phenotype distributions are not always Gaussian in the wild; in particular, they may be skewed (asymmetric). Formally, skewed distributions have a non-zero third central moment μ_3 , and skewness can be measured by scaling this moment by the standard deviation, $\alpha = \mu_3/\sigma^3$. As an example, in Table 1 we report phenotype distributions and phenotypic skewnesses for avian breeding phenology (i.e. date of first egg laid), a textbook example of phenotypic responses to climate change (Bradshaw and Holzapfel, 2008; Gienapp et al., 2014), which also includes several examples of the paradox of stasis in wild populations (Charmantier and Gienapp, 2014). We found a positive mean phenotypic skewness (across multiple years) in all seven populations of six bird species explored (table 1; skewness ranging from 0.40 in the Savannah Sparrow

Passerculus sandwichensis to 1.37 in the Common Tern *Sterna hirundo*).

Several non-exclusive processes may lead to a skewed phenotypic distribution, including: i) a sustained directional displacement of the adaptive optimum in time (Jones et al., 2012), such as the advancement of phenology with global warming (e.g. Visser et al., 2010); ii) migration between two populations with different adaptive optima (Débarre et al., 2015); iii) a skewed stabilizing fitness function (Urban et al., 2013). When phenotype distribution is skewed, it is sometimes possible to transform the trait to a scale that makes it close to Gaussian, for instance by log transformation, as is commonly done for morphological traits, notably when investigating allometry (e.g. Pélabon et al., 2013; Bolstad et al., 2015). However, transformation is not always recommended, as it may violate the natural scale allowing trait comparison, or lead to wrong biological interpretations (Houle et al., 2011). For some traits with specific non-normal distributions (e.g. Poisson), the response to selection may be analysed using the generalized linear model framework (de Villemereuil et al., 2016), but this is not applicable to traits with arbitrary distributions.

Previous theoretical work has explored the general response to selection of non-Gaussian traits (Burger, 1991; Turelli and Barton, 1990, 1994), and shown that a skewed distribution of breeding values can modify the expected response to selection. However, these studies did not consider a skewed distribution of environmental effects, and more generally did not address how phenotypic skewness affects empirical estimates of natural selection. We here investigate how skewed phenotype distributions affect natural selection and its estimation in wild populations. Specifically, we aim to understand whether the genetic versus environmental origin of phenotypic skewness has different effects on measurements of selection, and how these effects are captured by different metrics that reflect different strategies for quantifying selection. Our theoretical analysis, combined with simulations of empirical estimation with limited sample size, indicate that phenotypic skewness may have substantial effects on measurements of selection, and that the direction and magnitude of these effects depend on whether they concern the heritable or environmental components of phenotypic variation.

Table 1 Skewness of laying date in seven wild bird populations. Datasets are from the references listed in the last column, apart from the Kent Island Savannah sparrow data provided by Nathaniel T. Wheelwright. Laying date was annually standardised by the mean (i.e. laying date minus the annual mean laying date), in order to estimate the mean skewness of the laying date distribution on the entire dataset. Skewness was estimated with the function skewness from the R package Moment, and its significance tested using an Agostino test (function `agostino.test` of the same package). In the histograms, laying dates were binned by intervals of two days.

Species	Population	Date (number of years)	Number of measures (number of females)	Laying date Distribution (full line: mean, dotted line: median)	Skewness	Variance of skewness between years	References
<i>Passerculus sandwichensis</i>	Kent Island (Canada)	1987-2004 (18)	772 (NA)		0.40***	0.24	https://www.bowdoin.edu/kent-island/datasets/index.shtml
<i>Uria aalge</i>	Isle of May (Scotland)	1982-2005 (24)	2594 (NA)		0.40***	NA	Reed et al. 2006
<i>Strix uralensis</i>	Päijät-Häme (Finland)	1978-2008 (31)	1115 (361)		0.44***	NA	Brommer et al. 2012
<i>Cyanistes caerulescens</i>	Pirio (Corsica)	1980-2009 (30)	781 (489)		0.44***	0.33	Porlier et al. 2012
<i>Uria aalge</i>	Farallon Island (California)	1986-2005 (20)	583 (NA)		0.50***	NA	Reed et al. 2009; Schroeder et al. 2009
<i>Parus major</i>	Wytham, Oxford (England)	1961-2004 (44)	7784 (5068)		0.83***	0.48	Charmantier et al. 2008
<i>Sterna hirundo</i>	Wilhelmshaven (Germany)	1993-2008 (16)	1390 (294)		1.39***	0.40	Dobson et al. 2017

2. Model

2.1 Generalized response to selection

We focus on a quantitative trait, for which the phenotypic value z can be partitioned as

$$z = g + e \quad (1)$$

with g the breeding value contributing to parent-offspring resemblance – and hence to responses to selection – and e a residual component of variation (with mean 0) caused by environmental variation and developmental noise, as well as by non-additive genetic effects. Contrary to classical quantitative genetics (e.g. Lynch and Walsh, 1998; Falconer and MacKay, 1996), we do not assume that all components of the phenotype (z , g , and e) are normally distributed. In particular, we allow for non-zero third central moment μ_3 , and hence for skewness. The response to selection for a trait with arbitrary distribution of breeding values has been investigated by several authors (Burger, 1991; Turelli and Barton, 1990, 1994), who have shown that, up to third order,

$$\Delta \bar{z} = \sigma_g^2 \frac{\delta \ln(\bar{W})}{\delta \bar{z}} + \mu_{3g} \frac{\delta \ln(\bar{W})}{\delta \sigma_g^2}, \quad (2)$$

where μ_{3g} is the genetic third central moment. The first term in the right-hand side of equation (2) is the product of the directional selection gradient (defined as the derivative of log-mean fitness relative to the mean trait, see Table 2) by the additive genetic variance, as appears in the selection response of Gaussian traits (Lande, 1976a). In contrast, the second term only exists when the breeding value distribution is asymmetric, as it depends on μ_{3g} . The related component of selection is the partial derivative of natural logarithm mean fitness with respect to genetic variance.

2.2 Equilibrium mismatch with optimum

Here we are interested in modelling an evolutionary equilibrium caused by stabilizing selection, as often invoked to explain stasis in the long run (Arnold et al., 2001; Estes and Arnold, 2007; Hansen, 1997; Hansen and Houle, 2004). We model stabilizing selection using a quadratic function, where the absolute fitness $W(z)$ of an individual with phenotype z is

$$W(z) = W_{max} \left(1 - \frac{(z - \theta)^2}{2\omega^2} \right) \quad (3)$$

with W_{max} the maximal fitness values for an optimal phenotype θ , and ω the width of the fitness peak (stabilizing selection is stronger for smaller ω). This function is such that fitness declines with increasing distance between phenotype z and optimal values θ . A quadratic function is chosen here for mathematical convenience and because it is a good approximation to any form

of stabilizing selection under weak directional and quadratic selections, i.e. when the mean phenotype is close to the optimum and the phenotypic variance is smaller than the squared peak width. With this fitness function, the mean fitness of individuals with breeding values g , averaged over all possible phenotypic values accounting for environmental effects e , is

$$\tilde{W}(g) = W_{max} \left(1 - \frac{\sigma_e^2}{2\omega^2} - \frac{(g - \theta)^2}{2\omega^2} \right) \quad (4)$$

Hence with this fitness function, environmental effects affect the fitness of individuals with breeding value g only through their variance, but not their higher moments, including the environmental third order moment μ_{3e} . Averaging $\tilde{W}(g)$ over the distribution of breeding values g in the population, the mean fitness in the population is

$$\bar{W} = W_{max} \left(1 - \frac{\sigma_z^2}{2\omega^2} - \frac{(\bar{z} - \theta)^2}{2\omega^2} \right), \quad (5)$$

where $\sigma_z^2 = \sigma_g^2 + \sigma_e^2$ is the total phenotypic variance of the population, and $\bar{g} = \bar{z}$. Here again, the population mean fitness depends on the mean and variance of the phenotype, but not on the third central moment (or any higher moment) of the phenotypic distribution.

From equation (5), the directional selection gradient appearing in equation (2) (as derivative of mean fitness relative to mean phenotype, Table 2 and Lande, 1976b) is

$$\frac{\delta \ln(\bar{W})}{\delta \bar{z}} = -S(\bar{z} - \theta), \quad (6)$$

where

$$S = \left(\omega^2 - \frac{\sigma_z^2 + (\bar{z} - \theta)^2}{2} \right)^{-1}. \quad (7)$$

The parameter S is positive under weak stabilizing and directional selection ($\omega^2 > \frac{\sigma_z^2 + (\bar{z} - \theta)^2}{2}$), as required in the first place by the use of a quadratic fitness function. Equation (6) shows that directional selection acts as a restoring force towards the optimum (i.e. is in opposite direction to the deviation from the optimum $\bar{z} - \theta$), with a strength S that increases when the fitness peak is narrower (small ω), as shown by Lande (1976b). Furthermore, if the squared deviation from the optimum $(\bar{z} - \theta)^2$ is much smaller than phenotypic variance σ_z^2 (directional selection weaker than stabilizing selection), then

$$S \approx \frac{1}{\omega^2 - \frac{\sigma_z^2}{2}}, \quad (8)$$

such that directional selection is proportional to the deviation of the mean phenotype from the optimum in this model (since S no longer depends on $\bar{z} - \theta$).

The selection gradient on genetic variance appearing in equation (2) is, from equation (5),

$$\frac{\delta \ln(\bar{W})}{\delta \sigma_g^2} = \frac{-S}{2}. \quad (9)$$

This gradient is negative when selection is stabilizing (because stabilizing selection reduces genetic variance). Then combining equation (6) and (9) with equation (2), the response to selection under skewed phenotypic distribution and quadratic stabilizing selection is

$$\Delta \bar{z} = -S \left[(\bar{z} - \theta) \sigma_g^2 + \frac{\mu_{3g}}{2} \right]. \quad (10)$$

Note that equation (10) is exact, such that no higher-order term exists in the response to selection with this fitness function (Burger 1991). Assuming that the genetic variance and third central moment are maintained at an equilibrium between selection and other evolutionary forces including mutation, recombination, and possibly migration (see Introduction), the evolutionary equilibrium for the mean phenotype is obtained by setting $\Delta \bar{z} = 0$ in equation (10), leading to an equilibrium deviation from the optimum

$$(\bar{z} - \theta)_{eq} = -\frac{\mu_{3g}}{2\sigma_g^2}. \quad (11)$$

Hence, at the evolutionary equilibrium, when breeding values are skewed, the mean phenotype is not at the adaptive optimum. The distance between the mean phenotype and the optimum increases with the skewness of breeding values (equation 10). Importantly, even though there is a difference between the mean phenotype and the optimum, there is no response to selection since the population is at equilibrium.

2.3 Equilibrium selection gradient

Empirical estimates of directional selection gradients from finite population samples are generally not based on the relationship between population mean fitness and mean phenotype (as used above), but instead use regression of individual fitness on individual phenotype. For normally distributed traits, the linear version of the ordinary least-square (OLS) regression of relative fitness (i.e. individual fitness divided by mean fitness) on individual phenotype directly yields the slope of the natural log-mean fitness function ($\frac{\delta \ln(\bar{W})}{\delta \bar{z}}$), that is, the selection gradient used to predict the response to selection (Lande, 1976a; Lande and Arnold, 1983, and see table 2). This is no longer true when phenotype distributions are non-Gaussian, notably when they are skewed (as discussed in the Appendix of Lande and Arnold 1983, where a special rescaling of traits is introduced to correct for the effect of skewness). For instance, with a quadratic fitness function as modelled in equation (3), and using the fact that,

from equations (1), (3), (7) and (8), $\frac{W}{\bar{W}} - 1 = \left(\frac{S}{2}\right) \left[\sigma_z^2 - 2(\bar{z} - \theta)(z - \bar{z}) - (z - \bar{z})^2 \right]$, the selection gradient estimated by the OLS method is

$$\hat{\beta}_{OLS} = \sigma_z^{-2} E \left[(z - \bar{z}) \left(\frac{W}{\bar{W}} - 1 \right) \right] = -S \left[(\bar{z} - \theta) + \frac{\mu_{3z}}{2\sigma_z^2} \right], \quad (12)$$

where $E[\]$ denotes an expectation over the phenotypic distribution. Note that the gradient in equation (12) includes a contribution from *phenotypic* skewness (through the third central moment μ_{3z}). This shows that, if the phenotype distribution is skewed due to skewness in environmental effects distribution (i.e. $\mu_{3g} = 0$ but $\mu_{3e} \neq 0$, leading to $\mu_{3z} \neq 0$), the selection gradient $\hat{\beta}_{OLS}$ estimated by the Lande and Arnold (1983) method does not vanish when the mean phenotype is at the optimum (i.e. even if $\bar{z} = \theta$ at equilibrium because $\mu_{3g} = 0$). This occurs because the asymmetry in the phenotype distribution causes individuals to lie farther on one side of the optimum than on the other side, resulting in a non-zero directional gradient, when the fitness function is symmetric around the optimum (figure 1a). Note also the striking similarity between the response to selection in equation (10) and the measured selection gradient using OLS method in equation (12). The difference is that the former depends on the additive genetic variance and third central moment, while the latter depends on the phenotypic moments.

Table 2 Definitions of selection gradients. We here summarize alternative ways to conceptualize selection gradients, respectively as derivatives of (log) mean fitness with respect to mean phenotype (theoretical perspective, from Lande's extension of Wright's mean fitness landscape to quantitative traits), as average derivatives of individual relative fitness with respect to individual phenotype, or as ordinary least-square (OLS) regression slopes of individual relative fitness on individual phenotype. For directional selection, these expressions are all equivalent if the trait is normally distributed. Furthermore, the response to selection by the mean phenotype is proportional to these directional gradients only for a normally distributed trait.

	Derivative of mean fitness	Average Derivative of Individual Relative Fitness (ADIRF)	Regression slope (OLS)
Directional selection (Selection on trait mean)	$\frac{\partial \ln \bar{W}}{\partial \bar{z}} = \frac{1}{\bar{W}} \frac{\partial \bar{W}}{\partial \bar{z}} = \beta$	$\frac{1}{\bar{W}} E \left(\frac{\partial W}{\partial z} \right) = \beta$	$\frac{1}{\bar{W}} \frac{Cov(z, W)}{\sigma_z^2}$ (= β when z is Gaussian)
Stabilizing/disruptive selection (Selection on trait variance)	$\frac{\partial^2 \ln \bar{W}}{\partial \bar{z}^2} = \frac{1}{\bar{W}} \frac{\partial^2 \bar{W}}{\partial \bar{z}^2} - \left(\frac{1}{\bar{W}} \frac{\partial \bar{W}}{\partial \bar{z}} \right)^2 = \gamma - \beta^2$	$\frac{1}{\bar{W}} E \left(\frac{\partial^2 W}{\partial z^2} \right) = \gamma$	$\frac{1}{\bar{W}} \frac{Cov((z - \bar{z})^2, W)}{\sigma_z^4}$ (= γ when z is Gaussian)

Equation (12) can be used to predict the response to selection that would be estimated from a selection gradient estimated by OLS method, which is simply $\Delta\bar{z}_{OLS} = \hat{\beta}_{OLS}\sigma_g^2$. Comparison with the actual response to selection in equation (10) shows that

$$\Delta\bar{z}_{OLS} - \Delta\bar{z} = -\frac{S}{2}(h^2\mu_{3z} - \mu_{3g}). \quad (13)$$

Hence, the error made by ignoring phenotypic skewness when computing the response to selection as $\Delta\bar{z}_{OLS}$ is all the more important as the third central moment of breeding values μ_{3g} differs from the phenotypic third central moment μ_{3z} multiplied by the heritability h^2 . Finally, at evolutionary equilibrium, the selection gradient estimated by the OLS method according to Lande and Arnold (1983) is

$$\hat{\beta}_{OLS,eq} = -\frac{S}{2}\left(\frac{\mu_{3z}}{\sigma_z^2} - \frac{\mu_{3g}}{\sigma_g^2}\right). \quad (14)$$

Equation (14) shows that even at evolutionary equilibrium, where the mean phenotype no longer evolves in response to selection, the selection gradient measured by the OLS method (that is, using the classic Lande and Arnold (1983) approach) on a character with skewed phenotypic distribution is likely to be non-zero. The sign of the measured selection gradient depends on the origin of the phenotypic skewness: at evolutionary equilibrium, with environmental skewness, the selection gradient is of the opposite sign to the phenotypic skewness (figure 1a), while with genetic skewness, the selection gradient is of the same sign as phenotypic skewness (figure 1b). This occurs because phenotypic skewness affects the selection gradient by itself, but skewness of breeding values also affects the response to selection, displacing the mean phenotype from the optimum in the opposite direction.

2.4 Alternative measurements of selection

When phenotype distributions are skewed, the response to selection may still be correctly predicted empirically by using methods that do not assume normality. For instance, the Robertson–Price identity (Price, 1970; Robertson, 1966, 1968) states that the response to selection equals the additive genetic covariance between a trait z and relative fitness w , regardless of the phenotype distribution, that is, $\Delta\bar{z} = \sigma_a(z, w)$. Expanding terms in the covariance, using $\frac{\bar{W}(g)}{\bar{W}}$ (from equations (4) and (5)) as the additive genetic relative fitness associated with genotypes with breeding value g , after some simple algebra we recover the correct response to selection in equation (10).

A complementary approach would be to use a method that directly computes the theoretical selection gradients based on mean fitness. This strategy, originally introduced by Janzen and Stern (1998) for the specific case of logistic regression in viability selection, has recently been extended by Morrissey and Sakrejda (2013), who advocated its use in general, and proposed a framework based on generalized additive model (Wood, 2006, GAM, R package mgcv,

function gam) to directly estimate the theoretical selection gradient that appears in equation (2). More precisely, this method estimates the individual fitness landscape (relationship between individual traits and relative fitness), and uses it to compute the Average Derivative of Individual Relative Fitness (i.e. the mean slope of the individual fitness landscape, ADIRF method hereafter), which is equivalent to the derivative of mean fitness for the linear selection gradient (for the quadratic selection gradient, a correction term is added, Lande and Arnold, 1983 and table 2). This is implemented as `gam.gradient` in the `gsg` R package by Morrissey and Sakrejda (2013).

These alternative measurements of selection can be used in combination, in order to partition out the contribution of phenotypic skewness to selection and the response to selection. For instance, the part of the observed phenotypic selection attributable to skewness in phenotypes is provided by the difference between the selection gradients estimated by the OLS and ADIRF methods,

$$\hat{\beta}_{OLS} - \hat{\beta}_{ADIRF} = -\frac{S}{2} \frac{\mu_{3z}}{\sigma_z^2}. \quad (15)$$

Similarly, the part of the response to the selection attributable to skewness in breeding values is provided by the difference between the Robertson-Price identity and the response to selection predicted with the ADIRF method,

$$\Delta \bar{z}_{RP} - \Delta \bar{z}_{ADIRF} = \sigma_a(z, w) - \sigma_g^2 \hat{\beta}_{ADIRF} = -\frac{S}{2} \mu_{3g}. \quad (16)$$

Equation (16) holds even when the population is not at evolutionary equilibrium, but at equilibrium this term exactly cancels out the selective effect of the mismatch between \bar{z} and θ (equation 11). Note also that the component of the selection response in equation (16) depends on the genetic third central moment μ_{3g} , but not on heritability.

3. Simulation of selection estimation

To assess the relevance of these theoretical predictions for empirical estimates of selection with moderate sample sizes, we simulated data sets of skewed phenotypic distributions and associated fitness based on an adaptive landscape with a fitness optimum.

3.1 Simulation procedure

All simulations were performed in the R environment (R 3.2.4 version). Each individual phenotype was the sum of a breeding value and an environmental effect. Because skewness in phenotype distribution can have genetic or environmental origins, simulations were performed under two scenarios: i) skewed breeding values but normally distributed environmental effects; and ii) normally distributed breeding values but skewed environmental effects.

To explore a range of parameter values, we used a distribution that allows modifying skewness while controlling for mean and variance. We chose the

Gamma distribution $\Gamma(k, h)$ with shape and scale parameters k and h respectively, because its skewness is simply $\alpha = \frac{2}{\sqrt{k}}$. We made use of the properties relating the shape and scale parameters of a Gamma to its mean $\mu = kh$ and variance $\sigma^2 = kh^2$. Specifically, we chose the scale parameter as $h = \frac{\sigma}{\sqrt{k}}$ with σ the standard-deviation of the distribution, such that changing k changed skewness without changing the variance. Finally, adding $-kh$ to the variable (leading to a displaced Gamma distribution) allowed controlling for the mean.

In each simulation, the breeding value and environmental effect of each individual were drawn from distributions with the required moments. In particular, the distribution of environmental effects had mean zero in all simulations. The distribution of breeding values had mean zero in simulations with skewed environmental effects, while in simulations with skewed breeding values, the mean was displaced from the optimum according to equation (11). We used distributions with several theoretical values of skewness in breeding values or environmental effects: $\alpha = 0, 0.5, 1, 2, 3, 4$ or 5 . We only considered positive skewness, but all results can be transposed to negative phenotypic skewness, simply with opposite signs. When skewness was caused by the environmental effects, the latter had an expected variance $\sigma_e^2 = 1$, and we tested several variances of breeding values: $\sigma_g^2 = 1, \frac{1}{2}, \frac{1}{5}$ or 0 , leading to heritabilities $h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2} = \frac{1}{2}, \frac{1}{3}, \frac{1}{6}$ or 0 , respectively. Conversely, when skewness was due to breeding values, the genetic variance was set to $\sigma_g^2 = 1$, and the environmental variance varied: $\sigma_e^2 = 0, 1, 2$ or 5 , leading to heritability $h^2 = 1, \frac{1}{2}, \frac{1}{3}$ or $\frac{1}{6}$ respectively. Because the phenotype distribution is a mixture between the distributions of breeding values and environmental effects (one of which is Gaussian by assumption), its skewness is necessarily smaller (in absolute value) than the skewness of breeding values or environmental effects, and increases with increasing (respectively, decreasing) heritability (figure S1).

Selection was implemented using the quadratic fitness function in equation (3), with optimum $\theta = 0$, fitness maximum $W_{max} = 10$ and peak width $\omega = 5$. These values were chosen to be consistent with recent estimates for laying date in a wild great tit population (Chevin et al. 2015), so fitness W corresponded to the expected number of offspring per female reproductive event (i.e. selection on reproductive success). The actual offspring number for a female with phenotype z was randomly drawn from a Poisson distribution with mean $W(z)$, thus accounting for demographic stochasticity. Relative fitness was obtained by dividing absolute realized fitness (number of offspring) by the mean fitness in the population.

Directional and quadratic selection gradients were estimated in each simulation using the two methods described in the Model section: i) the classical OLS method of Lande and Arnold (1983), and ii) the ADIRF method, as implemented in the `gsg` R package by Morrissey and Sakrejda (2013). All selection gradients were standardized by variance – by multiplication by standard deviation or by variance, for directional or quadratic gradient respectively – in order to compare our results across parameter values, and with empirical studies. For every set of parameters, 100 replicates were performed and selection estimates obtained with both methods of estimation. We also estimated the

expected response to selection using the Robertson-Price identity (Robertson, 1968). For this purpose, we estimated the additive genetic values of fitness by calculating the mean expected fitness associated with each bin of breeding values of width 0.1. Note that knowing the exact breeding values of fitness and traits in our simulations allows for a much more accurate estimation of the Robertson-Price identity than would be possible in the wild, where these have to be estimated. We investigated two sample sizes frequently typically found in the literature: 200 (e.g. annual selection estimation, such as Reed et al. 2009) and 1000 individuals (e.g. long-term studies, such as Pelletier et al. 2007). However, because results were qualitatively similar, only results for the largest sample size (1000 individuals) are presented here.

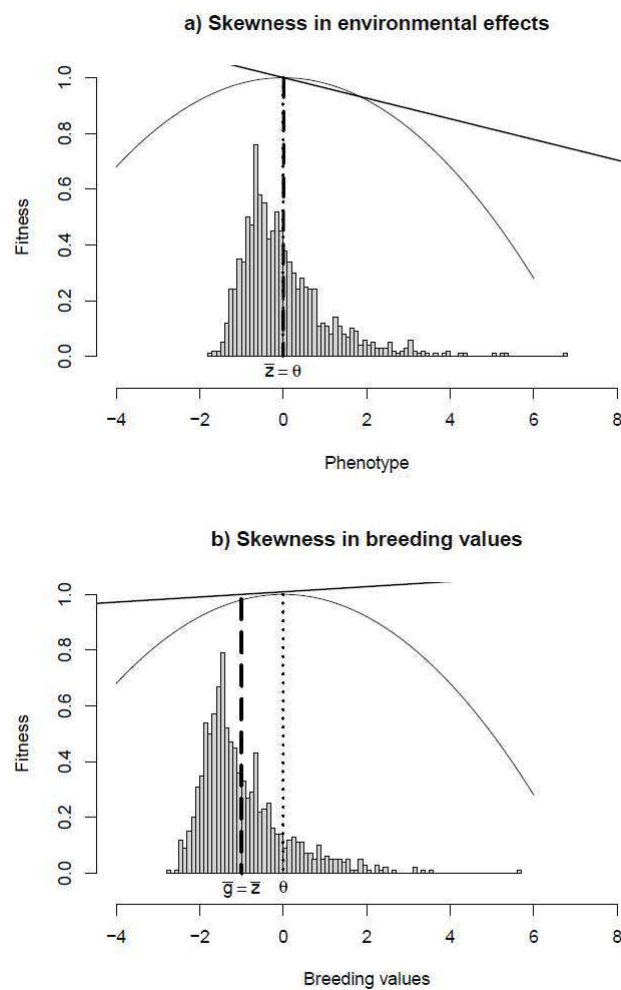


Figure 1: Influence of phenotypic skewness on estimates of directional selection. The histograms show phenotype distributions under skewed environmental effects (a) (skewness $\alpha_e = 2$, heritability $h = 0.1$), or skewed breeding values (b) (skewness $\alpha_g = 2$, heritability $h = 0.9$). The dotted line represents the optimum phenotype θ for the stabilizing fitness function (solid curve, equation (3)). The dashed line shows the mean phenotype. The solid straight line has a slope equal to the directional gradient estimated by OLS regression (as in Lande and Arnold (1983)).

3.2 Skewed environmental effects

When positive phenotypic skewness is only due to skewed environmental effects, the OLS method detects negative directional selection (figure 2a), that is, selection in the opposite direction to the skew, as predicted by our analytical developments. The strength of selection estimated by $\hat{\beta}_{OLS}$ increases with skewness in environmental effects, but decreases with increasing heritability (figure 2a). Directional selection gradients are detected with high power for moderate and high skewness (figure S2a). In contrast, the ADIRF method detects little or no directional selection (figure 2b and figure S2b for the power analysis), as expected because the mean phenotype is at the adaptive optimum in this case (since $\mu_{3g} = 0$), so the ADIRF estimation should be close to zero (equation 6). The part of selection detected by the OLS method that is attributable to phenotypic skewness ($\hat{\beta}_{OLS} - \hat{\beta}_{ADIRF}$, equation 15) is thus negative, with a magnitude that increases with increased skewness of environmental effects and decreased heritability (figure 2c), similar to $\hat{\beta}_{OLS}$. Estimates of quadratic selection are negative, as expected because of stabilizing selection, weakly sensitive to the skewness in environmental effects (figure S3), and quadratic selection is detected with high power (figure S4).

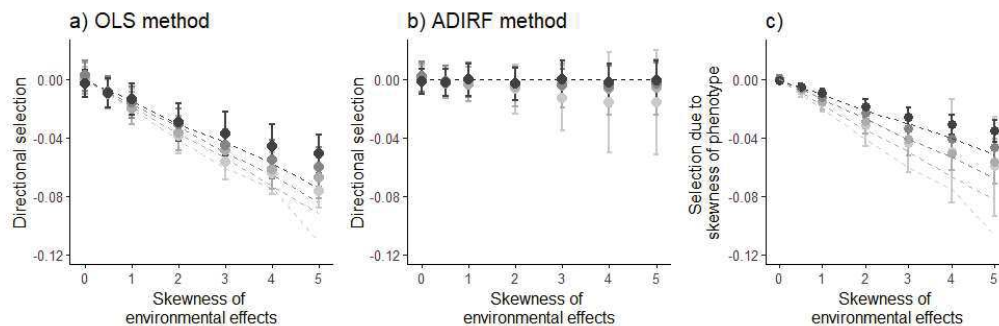


Figure 2: Estimated directional selection gradients with skewed environmental effects. Standardized directional selection gradient (mean \pm se) estimated by the OLS method (a) or the ADIRF method (b), and the contribution of phenotypic skewness to estimated selection with the OLS method (c), are represented against the skewness of environmental effects, for variable heritabilities ($h^2 = \frac{1}{2}; \frac{1}{3}; \frac{1}{6}$ or 0 from black to light grey). Dotted lines show predictions from the model, equation (14) for (a), 6-7-8 for (b), and (15) for (c).

3.3 Skewed breeding values

When phenotypic skewness is caused by skewed breeding values, both the OLS method and the ADIRF estimate positive selection gradients, that is, selection in the same direction as the phenotypic skew. This effect, which increases in magnitude when genetic skewness increases, is well predicted by our theoretical analysis (dashed lines in figure 3), and occurs because skewed breeding values cause a mismatch ($\bar{z} - \theta$) between the mean phenotype and the optimum at evolutionary equilibrium (equations 11-12). Selection is efficiently detected,

including for magnitudes of skewness comparable to our estimates from wild populations (table 1), and relatively small heritability (figure S5). Selection gradients estimated by the OLS method are smaller than those estimated using ADIRF (figure 3a,b), as a consequence of the contribution of phenotypic skewness to OLS gradients, in the direction opposite to the phenotypic skew. Indeed, the difference between the OLS and ADIRF estimates of directional selection are similar to that under environmental skewness (compare figure 2c and 3c). However, under skewed breeding value distribution, this component increases in absolute value with increasing heritability (figure 3c), since this causes larger phenotypic skewness (for a given skewness in breeding values).

The contribution of skewness in breeding values to the response to selection, as quantified by the difference between the Robertson-Price identity and the response to selection predicted by the ADIRF estimation ($\Delta\bar{z}_{RP} - \Delta\bar{z}_{ADIRF}$), is negative (i.e. opposite to the genetic skew), and increases in magnitude with increasing genetic skewness (figure 4). Note that at evolutionary equilibrium, this effect precisely cancels out the deviation of the mean phenotype from the optimum, and is in the opposite direction to the measured selection gradient in figure 3. As expected, the contribution of genetic skewness to the response to selection is well predicted by our model. Finally, the quadratic selection gradient depends little on skewness of breeding values distribution (figure S6), and is also significantly detected (figure S7).

4. Discussion and Conclusion

We show that skewness in phenotype distributions for a trait under selection for an optimum phenotype can have substantial impacts on natural selection and its estimation in the wild. With skewed environmental effects, the OLS method (i.e. the classic Lande and Arnold (1983) method) detects directional selection (in the opposite direction to the skew), even when the mean phenotype is at the optimum. In contrast, if phenotypic skewness is due, at least in part, to a skewed distribution of breeding values, the mean phenotype is displaced from the optimum at evolutionary equilibrium, because the curvature of the fitness function contributes to evolution of the mean phenotype when breeding values are skewed (Burger, 1991; Turelli and Barton, 1990, 1994). This displacement from the optimum contributes an additional term to the estimated directional selection, in the direction of the skew. In both cases (genetic or environmental skew), because the population is at equilibrium, there is no response to selection (i.e. the mean phenotype of the population does not evolve). Hence, selection would be detected on a heritable trait, but without any evolutionary response, so skewness in environmental effects (using OLS regression as in the Lande and Arnold (1983) method), and/or breeding values would contribute to the paradox of stasis.

According to classic meta-analyses (Hoekstra et al. 2001; Kingsolver et al. 2001; Kingsolver and Pfennig, 2007), estimates of standardized directional selection gradients are exponentially distributed with mean magnitude 0.23, which was recently re-evaluated to about 0.1 in a re-analysis of the same

dataset by Morrissey (2016), taking into account standard-error of estimates. Hence, estimated directional selection gradients in natural populations are of the same order of magnitude as directional selection generated by genetic or environmental skewness in our analysis (figure 2a,b and 3a,b). However, genetic (respectively, environmental) skewness can only contribute to an observed paradox of stasis when directional selection and genetic (respectively, environmental) skewness are of the same sign (respectively, of opposite signs). For laying date, negative directional selection is generally detected in wild populations (i.e. selection for earlier breeding, e.g. Sheldon et al. 2003; Porlier et al. 2012), while mean skewness tends to be positive (table 1). If phenotypic skewness of laying date is due to a skewed genetic values distribution, a positive (rather than negative) selection gradient will ensue at evolutionary equilibrium, so correcting for this effect would accentuate (rather than alleviate) the paradox of stasis. Conversely, if phenotypic skewness is mostly due to skewed environmental effects, it will contribute negatively to the selection gradient commonly estimated using ordinary least square regression (OLS, i.e. Lande and Arnold 1983), such that correcting for this effect would alleviate the paradox of stasis. However, the strength of selection would only slightly decrease: for a skewness of environmental effects of 1.5 – as can be observed in wild populations, table 1 – the strength of selection estimated by the OLS method would be around 0.03 (figure 2a,c), which is lower than the average estimate of selection gradients from meta-analyses (Kingsolver et al. 2001, Morrissey 2016).

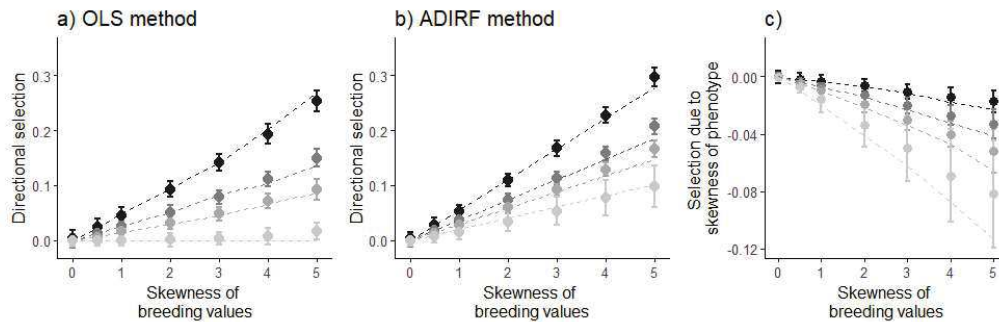


Figure 3: Estimated directional selection gradients with skewed breeding values. Standardized directional selection gradient (mean \pm se) estimated by the OLS method (a) or the ADIRF method (b), and the contribution of phenotypic skewness to estimated selection (c), are represented against the skewness of breeding values, for variable heritabilities ($h^2 = 1; \frac{1}{2}; \frac{1}{3}$ or $\frac{1}{6}$ from black to light grey). Dotted lines show predictions from the model, equation (14) for (a), 6-7-8 for (b), and (15) for (c).

Phenotypic distributions are a mixture of genetic and environmental effects, and it is empirically difficult to attribute phenotypic skewness to any of these sources using current quantitative genetic methods. Indeed, quantitative genetic methods, such as the ‘animal model’ that is commonly used for

natural populations (Kruuk, 2004), were designed to partition phenotypic variance into various genetic and non-genetic components, under the assumption of normally distributed traits (i.e. without skewness). It is unclear whether such methods can accurately partition genetic from non-genetic components of higher moments (such as the third central moment, contributing to skewness) for traits with non-Gaussian distributions. Nevertheless, phenotypic skewness and heritability could already be informative about the origin of the skew. For heritabilities of $1/3$ or less (as often reported for phenological traits), a phenotypic distribution with skewness $\alpha = 1$ (as seen in some wild populations, see table 1), if of genetic origin only, requires a skewness of breeding values distribution of about 10 (figure S1). This kind of distribution is probably unlikely in nature, and such a trait would perhaps not even be analysed using quantitative genetics, because of the extreme prevalence of genotypes with phenotypes near the mode. It is thus more likely that phenotypic skewness results from environmental effects, or a mixture of genetic and environmental skewness. This implies that skewness of laying date is more probably of environmental origin, and would thus have a contribution (albeit modest) to the paradox of stasis for this trait. A related crucial point is that when heritability is low, a strong skewness of breeding values can result in vanishingly weak skewness of phenotypic distribution (figure S1). Hence, even when skewness is essentially undetectable at the phenotypic level, the population mean phenotype can be displaced from the adaptive optimum at evolutionary equilibrium, thereby generating directional selection.

We have shown that taking into account the skewness in the distributions of phenotypic components of quantitative traits can help understand evolutionary dynamics in the wild, and could partially alleviate the paradox of stasis in some cases. Because the OLS method by Lande and Arnold (1983), when applied in its classic form, does not necessarily predict the correct response to selection for characters with a skewed distribution (as indeed highlighted by Lande and Arnold (1983), who proposed a possible correction in their Appendix), we encourage the use of a combination of approaches, including the Robertson-Price identity and methods based on derivatives of mean fitness derived from the individual fitness function, when investigating natural selection on skewed phenotypic traits.

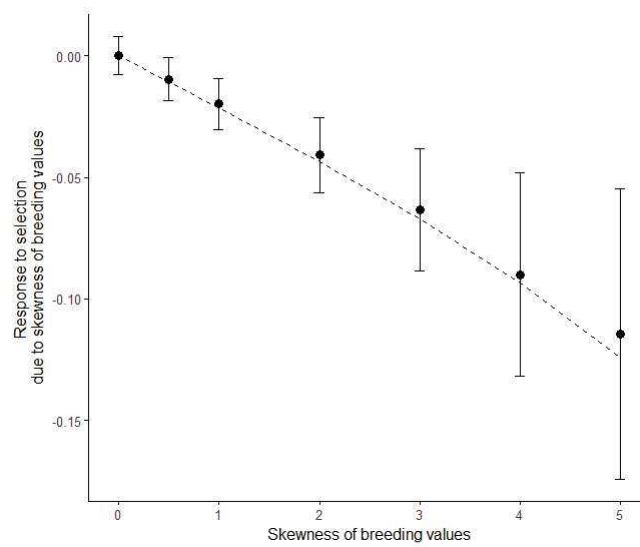


Figure 4: Contribution of skewed breeding values to the selection response. The difference between the response to selection predicted by the Robertson-Price identity versus using the selection gradient estimated by the ADIRF method is shown for simulations (mean \pm se), together with the theoretical prediction in equation (16) in dashed lines, for the same simulations as used in figure 3.

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Data accessibility

The doi for our data is <https://doi.org/10.5061/dryad.pt07g>.

Author's contributions

CT, AC, PAC and LMC conceived the study. SB and LMC conducted the analyses. SB, LMC, CT, AC and PAC wrote the manuscript.

Competing interests

We have no competing interests.

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Supplementary materials

Figure S1

Skewness in phenotypic distribution against skewness in breeding values. Mean estimations of skewness and their standard errors in simulations (1000 individuals) are shown for variable heritabilities ($h^2 = 1; \frac{1}{2}; \frac{1}{3}$ or $\frac{1}{6}$ from black to light grey respectively). The dotted lines plots $y = x$. Typical distributions with skewness $\alpha = 0, 1, 2, 5$ and 10 , are illustrated below the graph. Note that confidence intervals increase with expected skewness (parameter of the Gamma distribution). This is because realized skewness becomes more variable when samples are drawn from distributions with higher expected skewness, as a result of the increased probability to not pick values at the right tail of the distribution.

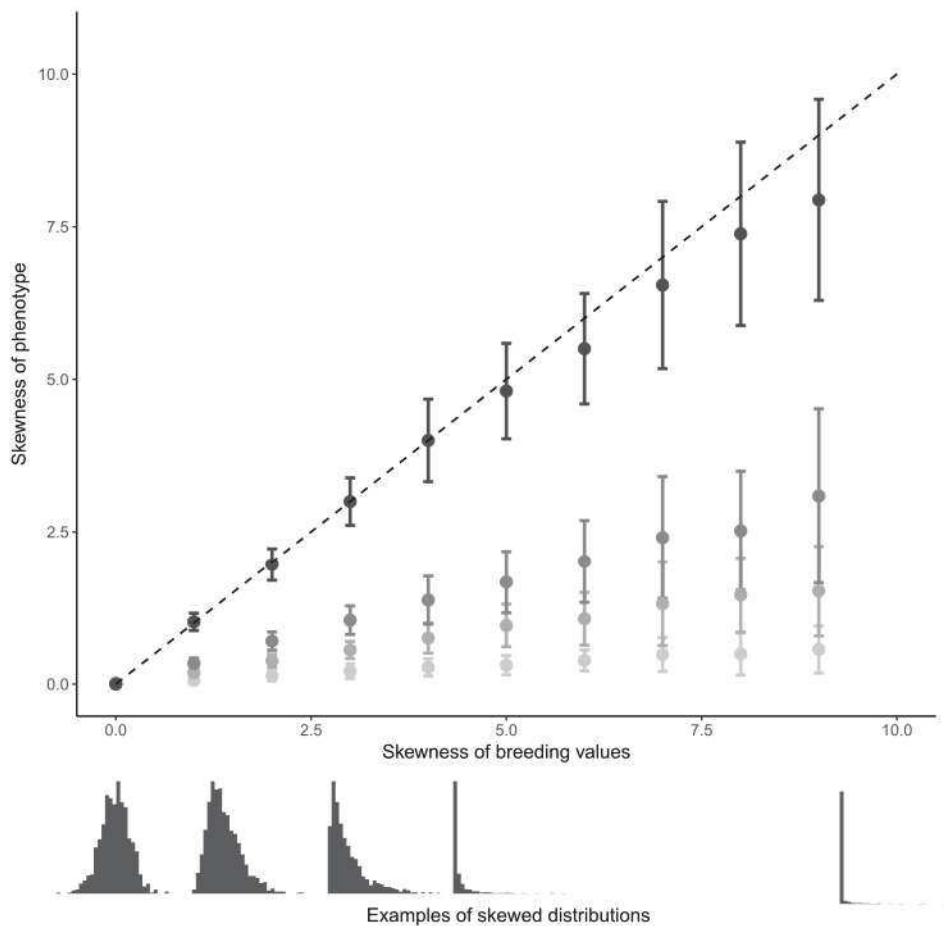


Figure S2

Power to detect directional selection gradients under skewed environmental effects. The proportions of simulations where a significant selection gradient was estimated by the OLS method (a) or the ADIRF method (b) are shown for several combinations of skewness of environmental effects and heritability. In black, positive selection gradients; in grey, negative selection gradient. At the top of each frame, values of heritability: $h^2 = 0, \frac{1}{6}, \frac{1}{3}$ or $\frac{1}{2}$. Significance of selection gradients from zero is determined by a Student test at 5% (a) or a permutation test 1000 permutations) (b).

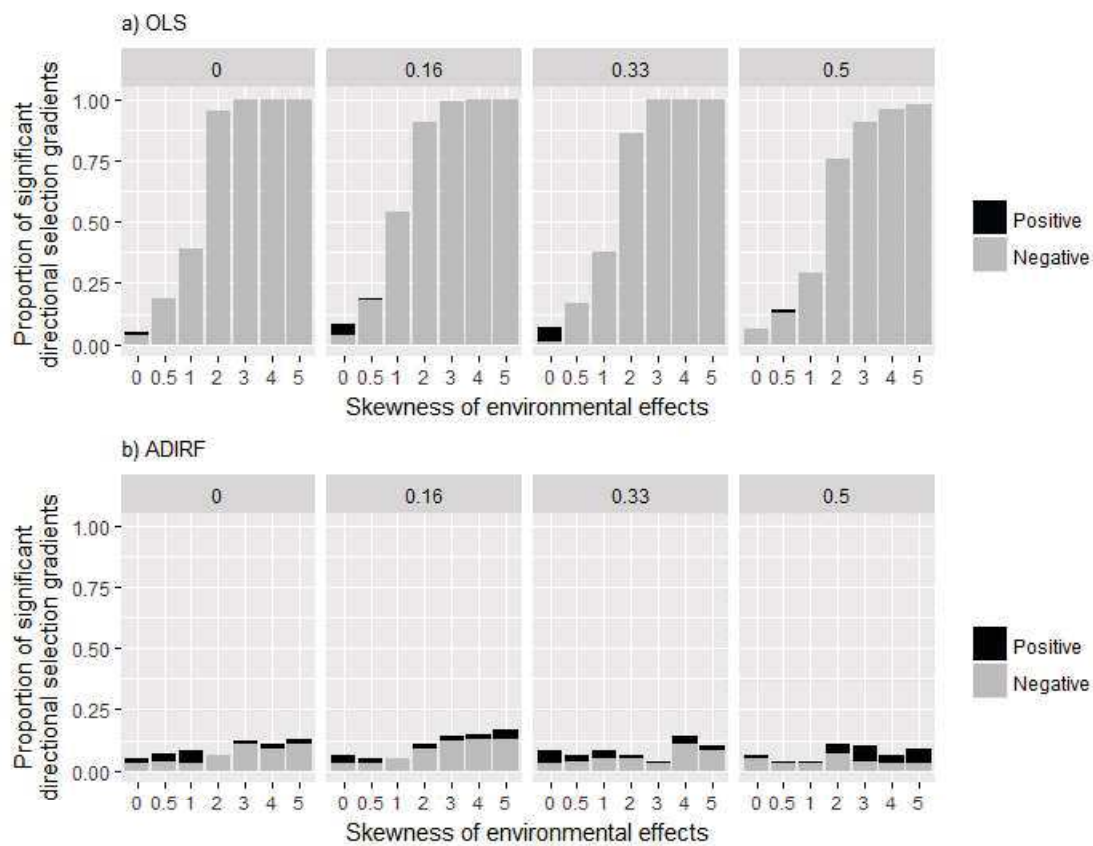


Figure S3

Estimated quadratic selection gradients with skewed environmental effects. Standardized quadratic selection gradient (mean \pm se) estimated by the OLS method (a) or the ADIRF method (b), are represented against the skewness of environmental effects, for variable heritabilities ($h^2 = \frac{1}{2}; \frac{1}{3}; \frac{1}{6}$ or 0 from black to light grey). Dotted lines show predictions from the model, using $\hat{\gamma} = \delta^2 \ln(\bar{W}) / \delta \bar{z}^2 + \beta^2$ (Lande and Arnold (1983)) and the equation (14) or equation 6-7-8 for (a) and (b) respectively (see table 2).

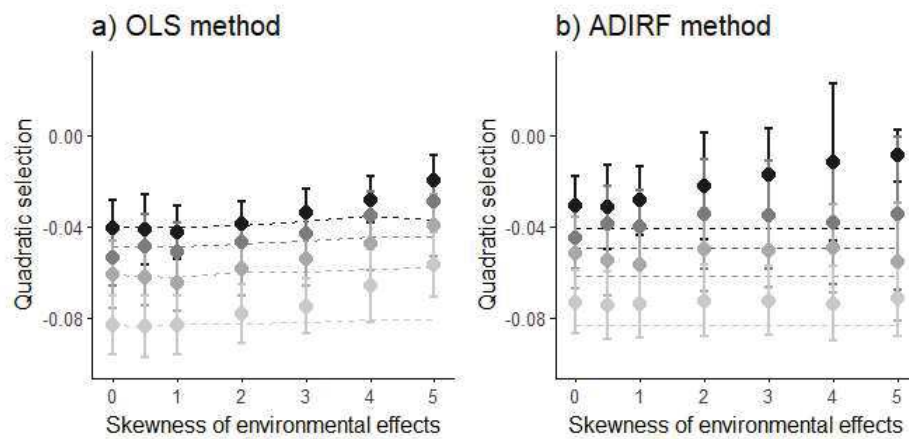


Figure S4

Power to detect quadratic selection gradient under skewed environmental effects. The proportions of simulations where a significant selection gradient was estimated by the OLS method (a) or the ADIRF method (b) are shown for several combinations of skewness of environmental effects and heritability. In black, positive selection gradients; in grey, negative selection gradient. At the top of each frame, values of heritability: $h^2 = 0, \frac{1}{6}, \frac{1}{3}$ or $\frac{1}{2}$. Significance of selection gradients from zero is determined by a Student test at 5% (a) or a permutation test (1000 permutations) (b).

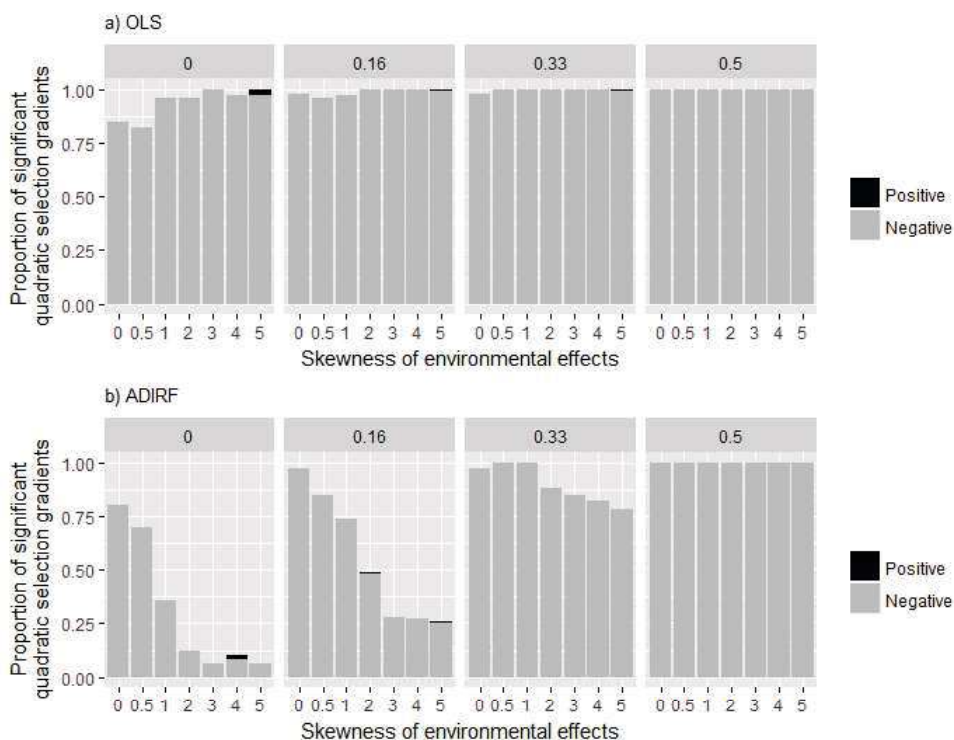


Figure S5

Power to detect directional selection gradient under skewed breeding values. The proportions of simulations where a significant selection gradient was estimated by the OLS method (a) or the ADIRF method (b) are shown for several combinations of skewness of breeding values and heritability. In black, positive selection gradients; in grey, negative selection gradient. At the top of each frame, values of heritability: $h^2 = \frac{1}{6}, \frac{1}{3}, \frac{1}{2}$ or 1. Significance of selection gradients from zero is determined by a student test at 5% (a) or a permutation test (1000 permutations) (b).

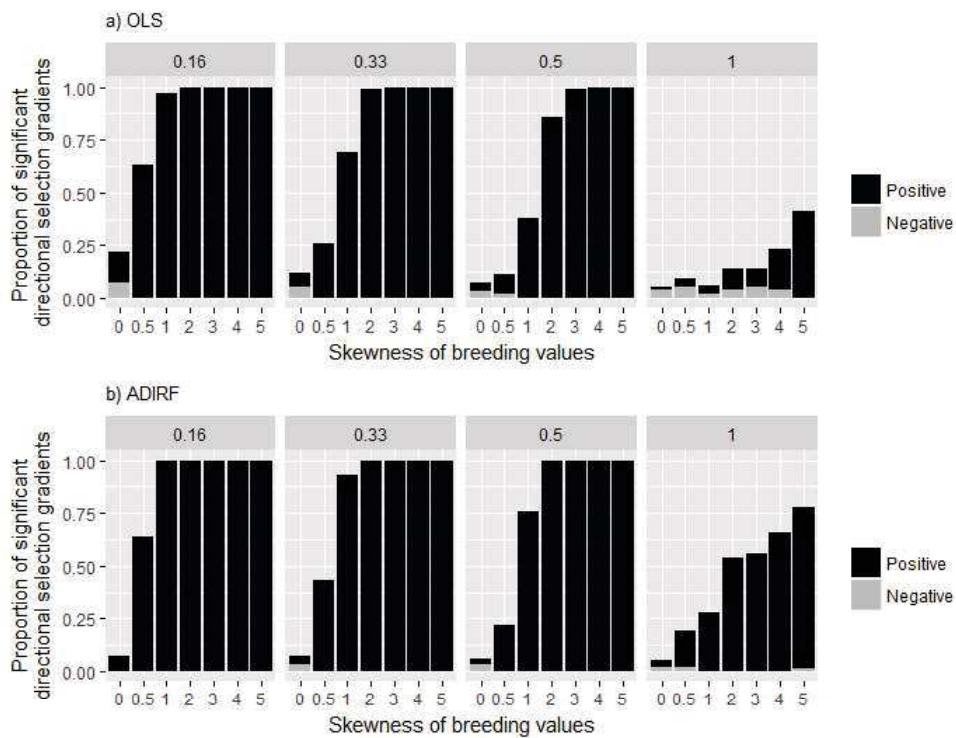


Figure S6

Estimated quadratic selection gradients with skewed genetic effects. Standardized quadratic selection gradient (mean \pm se) estimated by the OLS method (a) or the ADIRF method (b), are represented against the skewness of breeding values, for variable heritabilities ($h^2 = 1; \frac{1}{2}; \frac{1}{3}$ or $\frac{1}{6}$ from black to light grey). Dotted lines show predictions from the model, using $\hat{\gamma} = \delta^2 \ln(\bar{W}) / \delta z^2 + \beta^2$ (Lande and Arnold, 1983) and the equation (14) or equations 6-7-8 for (a) and b) respectively (see table 2).

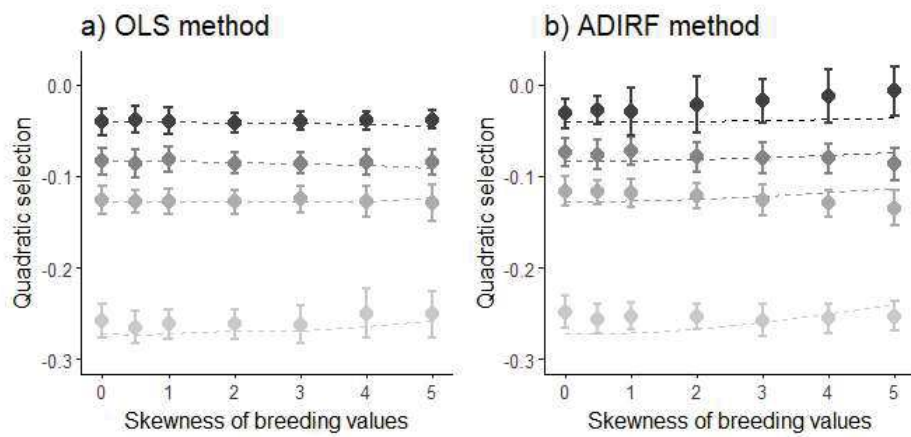
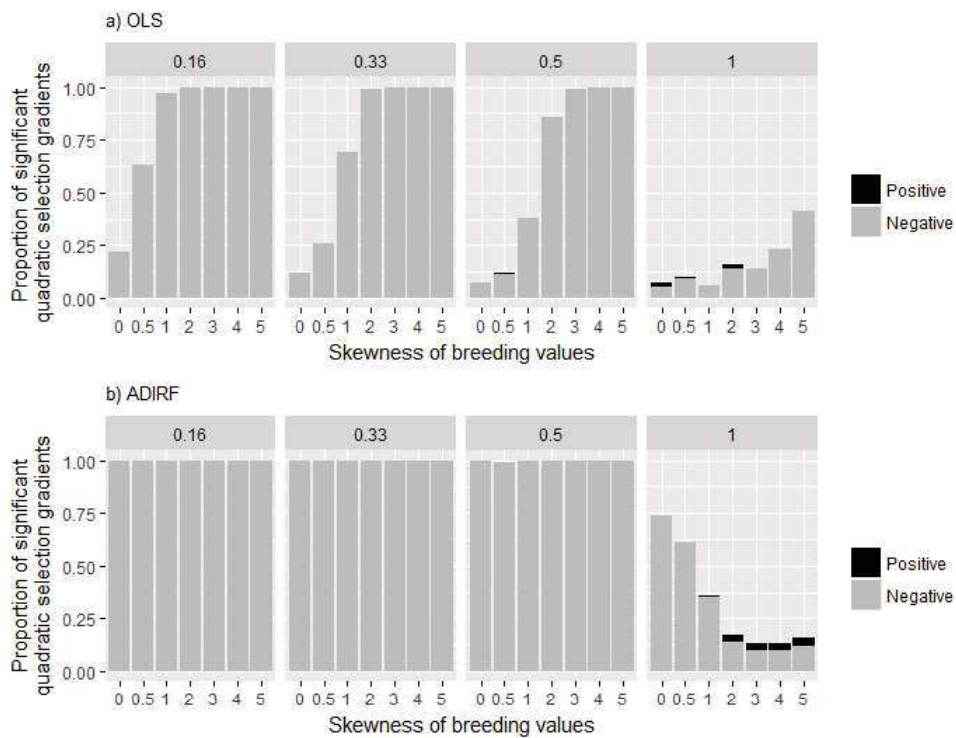


Figure S7

Power to detect quadratic selection gradients under skewed breeding values. The proportions of simulations where a significant selection gradient was estimated by the OLS method (a) or the ADIRF method (b) are shown for several combinations of skewness of breeding values and heritability. In black, positive selection gradients; in grey, negative selection gradient. At the top of each frame, values of heritability: $h^2 = \frac{1}{6}, \frac{1}{3}, \frac{1}{2}$ or 1. Significance of selection gradients from zero is determined by a Student test at 5% (a) or a permutation test (1000 permutations) (b).





Hike to Girolata (Corsica).

(Credits: Anne Charmantier)

MANUSCRIPT E

Selection and evolution of plasticity in a fluctuating natural environment

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Manuscript in preparation

This manuscript presents preliminary results, and complementary analyses are still needed to complete the study. Importantly, propagating estimation errors in the different steps make the analyses more complex and time consuming. Confidence intervals have thus not yet been taken into account in all analyses and graphical representations; but they will be included in all analyses before any submission to a scientific journal.

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Abstract

Organisms live in environments that vary in time with different magnitude, speed, and predictability. Current climate change is altering the patterns of these fluctuations, potentially modifying selective regimes in natural populations. In particular, selection on phenotypic plasticity is expected to strongly depend on patterns of environmental fluctuations, notably the predictability of selection by the environmental cue inducing a plastic response. This in turn affects the evolution of plasticity, if it is heritable. However, there is few empirical evidence of selection on plasticity in wild populations, and even scarcer evidence of fluctuating selection on and response to selection of plasticity. Using a long-term monitoring of wild Mediterranean blue tits in Corsica, we estimate annual selection on plasticity, as well as potential costs of plasticity, from individual measurement of fitness and traits combined with environmental time series. We detect a fluctuating environment in our study site, and our results are indicative of temporally variable natural selection on both laying date and its plasticity. Our preliminary results also suggest that both reaction norm intercept and slope may respond to fluctuating selection across years, and that these changes are well predicted by the multivariate breeder's equation for the intercept, but not for not the slope of the reaction norm.

*Key words: Reaction norm, Phenotypic plasticity, Natural selection, I^*E , G^*E , Laying date, Wild blue tits*

1. Introduction

Organisms live in environments that vary through time at multiple scales, from cyclical seasonal and diurnal oscillations to long-term trends such as global warming. In addition to such deterministic changes, essentially all environments display stochastic noise, that is, random fluctuations that can only be predicted probabilistically. This stochastic component of environmental variation is characterized not only by its magnitude – for instance, the variance of its stationary distribution – but also by its temporal autocorrelation pattern, which quantifies the extent to which one time point is a good predictor of the next (Vasseur and Yodzis, 2004). The ongoing climate change is expected to affect not only the mean of environmental variables such as temperature, but also their temporal variance and (auto)correlation structure (Wigley et al., 1998; Boer, 2009).

Theory of adaptation to randomly changing environment has shown that stochastic environmental fluctuations can have a strong impact on eco-evolutionary dynamics (Lande and Shannon, 1996; Ashander et al., 2016; Chevin et al., 2017). Species that cannot disperse have to cope with local environmental fluctuations via different non-exclusive mechanisms, depending on the frequency and predictability of environmental variation (Botero et al., 2015; Tufto, 2015). When environments fluctuate on time scales much larger than a generation (coarse-grained variation), the mean phenotype in a population can track the value favoured by environmental conditions via evolutionary responses to natural selection, provided there is genetic variation (microevolution). A strategy to ‘spread the risk’, such as bet-hedging, can be favoured when the environmental variability is unpredictable. In contrast, when environmental variation can be predicted on a time frame close to a generation, phenotypic plasticity, the expression of different phenotypes by a given genotype in response to environmental conditions (Travis, 1994; Pigliucci, 2001; DeWitt and Scheiner, 2004; Brommer, 2013), will be advantageous (Botero et al., 2015; Tufto, 2015). If phenotypic plasticity is heritable (as shown empirically in the wild in few case studies, e.g. Nussey et al., 2005; Husby et al., 2010), it can then evolve to an adaptive level such that plastic responses contribute to tracking of the phenotype favoured by the selection across environments (Gavrilets and Scheiner, 1993; Lande, 2009; Tufto, 2015). In addition, evolvable phenotypic plasticity may also fluctuate to some extent in response to environmental fluctuations, and this too may contribute to tracking of natural selection through time (Tufto, 2015). However, in contrast to this abundance of theoretical predictions, only a few empirical studies have estimated selection on phenotypic plasticity in natural populations (e.g. Nussey et al., 2005; Pelletier et al., 2007; Ramakers et al., 2018; Arnold et al., 2019), and we found little empirical test of temporal changes in selection on plasticity, nor for responses to selection of plasticity in nature. The evolution of plasticity in fluctuating environment and its role in the adaptation to environmental variability *in natura* is thus for now still largely unknown.

Phenotypic plasticity is increasingly shown to be a major component of observed phenotypic responses to climate change (Gienapp et al., 2007; Merilä and Hendry, 2014; Hoffmann and Sgro, 2011). Still the question often remains whether current plastic responses are enough to track the phenotype favoured by natural selection across environments, enabling population persistence (Phillimore et al., 2016; Radchuk et al., 2019), or whether evolution, including the evolution of plasticity, will be required. Furthermore, since climate change is altering the (auto)correlation pattern of environmental variables (Wigley et al., 1998; Boer, 2009), and thereby the predictability and reliability of cues inducing plastic responses, the selective pressure on plasticity in response to environmental fluctuations is likely to be modified, and we need to understand how and how much. To answer these questions, we need to estimate natural selection on reaction norms through time, and their potential for responses to selection, in a naturally fluctuating environment.

How to measure selection on phenotypic plasticity and assess its adaptiveness are deceptively simple questions. Conceptually, an adaptive plastic response is one that produces the adequate phenotype in each environment (Ghalambor et al., 2007). The benefit of plasticity thus comes from the way plastic responses relate to selection on the expressed phenotype across environments. In other words, the component of selection on plasticity that matters for adaptation to a changing environment is indirect selection (*sensu* Lande and Arnold, 1983), via the covariance of phenotypic plasticity with the expressed trait across environments. This is what determines how plasticity evolves in theoretical models, in the absence of costs (de Jong, 1990; Gavrilets and Scheiner, 1993; Lande, 2009). However, even when phenotypic plasticity is adaptive for its effect on the expressed trait across environments, it may be costly *per se*, notably because of maintenance costs, the cost of acquiring information about the environment, or a covariation of plasticity with developmental instability for example (van Tienderen and Koelewijn, 1994; Dewitt et al., 1998). Such costs of plasticity are conceptually defined, and empirically estimated, as the decrease in fitness caused by the plastic capacity of an individual, after taking into account the fitness effect of producing different phenotypes in different environments (van Tienderen and Koelewijn, 1994; Dewitt, 1998; Dewitt et al., 1998; Scheiner and Berrigan, 1998; Van Buskirk and Steiner, 2009; Auld et al., 2010). The reciprocal cost of being less plastic is described as a cost of canalization (Van Buskirk and Steiner, 2009). It is noteworthy that some authors proposed estimating selection on plasticity via the relationship between fitness and phenotypic plasticity correcting for selection on the expressed phenotype/reaction norm intercept (the ‘bivariate method’ to estimate selection on plasticity, e.g. Brommer et al., 2012; Arnold et al., 2019), while others used seemingly similar approaches to estimate the cost of plasticity (e.g. Van Buskirk and Steiner, 2009). This warrants a homogenization effort, in order to facilitate practical comparison of results across types of studies (natural populations, laboratory) and organisms. Here we address this issue in the context of continuous reaction norms under a fluctuating natural environment.

Phenology is among the strongest phenotypic response to climate change in terrestrial organisms (Parmesan, 2006). In particular, laying date variation with respect to spring temperature in temperate insectivorous birds is one of the most studied examples of phenotypic plasticity. In many passerines from temperate regions, reproductive phenology varies among years, mainly according to early spring temperature variation: birds lay on average earlier in warmer springs (e.g. Phillimore et al., 2016). The reaction norm of laying date with respect to spring temperature is generally close to linear, with a negative slope that quantifies plasticity. Laying date is a repeated trait in most passerines, as females lay in general (at least) one clutch per year. This allows estimating reaction norms for each individual, based on several phenotypic observations of each individual in different environments, in contrast to developmental plasticity for which different groups of related individuals have to be measured across environments (Scheiner and Lyman, 1989). A pedigree can then be used to partition additive genetic and environmental components of variance in individual reaction norms.

The reason why reproductive timing is crucial for annual reproductive success in many passerine populations is because the phenology of insect preys that parents use to feed chicks also varies among years in response to environmental variables (e.g. Dunn, 2004; Salis, 2015). Therefore, birds that synchronize their reproductive phenology with the phenology of their preys have higher reproductive success (e.g. Reed et al., 2013). Climate change is causing changes in bird and insect phenologies (e.g. Visser et al., 2006), inducing mismatches between the two trophic levels, which can have dramatic consequences for population dynamic and persistence (Visser and Gienapp, 2019). These phenological changes in the trophic chains are potentially changing selective pressures on laying date and its plasticity, and hence their responses to selection. We studied variation of directional selection on laying date in a wild blue tit population studied for more than forty years, and explored how this translates into selection and response to selection of laying date plasticity. Furthermore, we partitioned selection on plasticity into a component due to the expressed trait, and a component reflecting the potential cost to being plastic.

2. Material and Methodes

2.1 Field management and data collection

We studied a natural population of blue tits (*Cyanistes caeruleus ogliastrae*), a small insectivorous and cavity-nesting passerine. This population is located in the ‘E-Pirio’ forest (42°34’N, 08°44’E), in the Mediterranean island of Corsica, and has been monitored for about forty years (Blondel et al., 2006; Charmantier et al., 2016). Bird reproduction is followed every spring: for each of about 200 nest-boxes, breeding adult birds and their chicks are fitted metal rings with unique numbers, and for each breeding attempt the laying date (day when the first eggs of the first clutch of the year per female is laid), clutch

size and number of fledglings are recorded. The overall data set included 1696 reproductive attempts for 851 females (see supplementary material 1 for sample sizes per year). Birds are aged thanks to their plumage (giving exact age of yearling), or using the number of yearly-observations of identified individuals (giving minimum age for individuals older than 1 year, c. 40% of the population). In addition to bird phenology, we follow the date of peak in the abundance of caterpillars, which are the main prey for chicks (Blondel et al., 1991). This is done by collecting frass falling from trees during the whole reproductive season, and estimating the peak in caterpillar abundance as the maximum of the distribution of abundance over time. Temperature records come from the French national meteorological station of Saint Martin de Londres (< 18 km of the studied site) and are well correlated to the local temperature in the study site (Bonamour et al. 2019).

2.2 Theoretical predictions and statistical analyses

Selection on reaction norm

Estimating selection on reaction norm

The first step to estimate selection on reaction norm, which has been implemented by previous authors (van Tienderen and Koelewijn, 1994; Ramakers et al., 2018), consists of characterizing selection on reaction norm components of laying date based on selection on laying date itself. Consider a repeated plastic phenotypic trait z with linear reaction norm, with intercept a and slope b that may vary among individuals, such that the phenotype of individual i exposed to the environmental cue E_j in year j is

$$z_{ij} = a_i + b_i E_j + \epsilon_{ij} \quad (1)$$

where ϵ_{ij} is a residual component of phenotypic variation for individual i in year j . The reaction norm parameters can be treated as two traits, and written in vector form as $\begin{pmatrix} a \\ b \end{pmatrix}$ (where we have omitted indices i for simplicity). The directional selection gradient β on any normally distributed trait is defined theoretically as the partial derivative of (log) mean fitness with respect to mean phenotype for this trait (Wright, 1937; Lande, 1976). If a and b are normally distributed (with mean \bar{a} and \bar{b}), so is z , and from the chain rule, the directional selection gradients on reaction norm parameters in year j are

$$\begin{pmatrix} \beta_{a,j} \\ \beta_{b,j} \end{pmatrix} = \beta_{z,j} \begin{pmatrix} \frac{\delta \bar{z}_j}{\delta \bar{a}} \\ \frac{\delta \bar{z}_j}{\delta \bar{b}} \end{pmatrix} = \beta_{z,j} \begin{pmatrix} 1 \\ E_j \end{pmatrix} \quad (2)$$

where $\beta_{z,j}$ is the selection gradient on the expressed mean phenotype \bar{z}_j in year j . Equation (2) shows that the selection gradient $\beta_{b,j}$ on reaction norm slope, or plasticity, in year j equals the selection gradient $\beta_{z,j}$ on the expressed trait – here laying date – in that year, multiplied by the value of the environmental

cue E_j inducing the plastic response by this trait. However, such selection gradients on plasticity may be difficult to interpret, as they depend on the environment, which has arbitrary units, and may not relate in an obvious way to the change in plasticity under selection (see section below on evolution of reaction norms). Alternatively, we can estimate selection differentials from the product of the multivariate selection gradient by the phenotypic covariance matrix of reaction norms, to directly yield results in terms of trait change (for plasticity and reaction norms intercept).

Annual selection gradients $\beta_{z,j}$ on laying date were estimated in our blue tit population as regression slopes of annual relative reproductive success – here approximated by the number of fledglings per female per breeding attempt (we only include the first broods in the analyses) – against standardized laying dates (mean-centered laying date scaled to its standard deviation per year, Lande and Arnold, 1983). The environmental cue E_j is the average daily mean temperature during the time windows most correlated with the among-year variation of the laying date, i.e. between March 31th and May 7th (Bonamour et al., 2019). The environmental cue E_j was mean-centered over the study period in all analyses. We ran models to estimate selection gradients on laying date for each year independently.

Partitioning selection on reaction norm

Phenotypic plasticity is expected to evolve when the environmental cue that triggers the plastic response allows predicting which phenotype will be favoured by natural selection. In other words, the environmental cue should influence the relationship between the trait and fitness of individuals. Consider the situation where temporal variation in the selection gradient β_z on the expressed trait is partly predicted by the environmental cue E_j . Then we may write for selection on the expressed phenotype z at time j , $\beta_{z,j} = \beta_{E,0} + \beta_E E_j + e_{\beta,j}$, where the slope β_E quantifies the change in directional selection with respect to the environmental cue. $e_{\beta,j}$ is a residual component of temporal variation in selection on the expressed trait. Then from equation 2, the selection gradient on plasticity becomes $\beta_{b,j} = (\beta_{E,0} + \beta_E E_j + e_{\beta,j}) E_j$. In a temporally changing environment, the expected selection gradient on plasticity across the study period, assuming the environmental cue is mean-centered, is thus

$$\bar{\beta}_b = \beta_E \sigma_E^2. \quad (3)$$

Hence, the mean selection strength of plasticity over time increases with the variance in the cue, and the slope of selection on the trait with respect to the cue.

We estimated the extent to which the environmental cue for plasticity is a good predictor of the relationship between annual reproductive success and laying date using the following model

$$W_{ij} = \mu + \beta_{E,0}LD_{ij} + \alpha_E E_j + \beta_E(LD_{ij}E_j) + \alpha_A A_{ij} + \alpha_{A^2} A_{ij}^2 + Y_j + I_i + \epsilon_{ij} \quad (4)$$

where the relative annual reproductive success W_{ij} of the female i in the year j is explained by its laying date LD_{ij} and the annual environmental cue E_j . The interaction between laying date and temperature (β_E) allows estimating how the trait – fitness correlation changes with environmental cue, and hence the extent to which selection on the trait can be predicted by the cue E_j . We also added age A_{ij} and age squared A_{ij}^2 as fixed effects, because fecundity is influenced by female age in this population (Bonamour et al. submitted). We included two random effects (Y_j and I_i) to take into account the non-independence of observations on the same females and during the same years.

The ‘bird – prey time lag’ as a cause of selection

Even though phenotypic plasticity is only favoured in response to a cue that is a good predictor of (future) phenotypic selection, the cue itself is generally not the cause of selection. This is clear for most passerine phenology in temperate region, where the most direct measurable cause of selection is the match between avian breeding phenology and the prey peak date of abundance (chicks are mainly fed by caterpillars in our blue tit population, Blondel et al., 1991), and somewhat more indirectly the temperature cue that triggers caterpillar phenology. Denoting as U the expected causal selective environment (*sensu* Wade and Kalisz, 1990; MacColl, 2011) – here the distance between laying date and caterpillar peak date of abundance of the year – temporal variation in selection on the expressed trait across years can be partitioned into a part related to the selective environment β_U , and residual component $e_{\beta,U}$ of variation, leading to $\beta_z = \beta_{U,0} + \beta_U U + e_{\beta,U}$. Then, the expected selection gradient over time becomes, if the causal environment and the cue are mean-centered

$$\bar{\beta}_b = \beta_U \text{cov}(U, E) + \text{cov}(e_{\beta,U}, E) \quad (5)$$

The average selection on plasticity thus depends on the slope of the selection gradient on the measured cause of selection, multiplied by the covariance between this cause of selection and the cue. The second term in the sum is covariance of the cue with other unmeasured causes of selection that contribute to $e_{\beta,U}$.

We estimate the influence of the ‘bird – prey time lag’ (U_{ij}) on laying date selection as

$$W_{ij} = \mu + \beta_{U,0}LD_{ij} + \alpha_U U_{ij} + \beta_U(LD_{ij}U_{ij}) + \alpha_A A_{ij} + \alpha_{A^2} A_{ij}^2 + Y_j + I_j + \epsilon_{ij} \quad (6)$$

The ‘bird – prey time lag’ U_j was defined as the yearly individual distance between the laying date and the annual caterpillar peak date of abundance

(laying date minus caterpillar peak date). Note that highest food requirements of chicks occurs 9 days after hatching, and mothers lay one egg a day and incubate these eggs for 14 days, such that the optimal laying date resulting in an expected perfect match between the bird and prey phenologies when the ‘bird – prey time lag’ about -30 days, for a clutch of 7 eggs (average clutch size of E-Pirio population is 6.6 eggs).

In summary, by measuring β_E (equation 3), β_U (equation 5), σ_E^2 , and $cov(U, E)$, we can partition the ways by which selection operates on plasticity, via its influence on the expressed phenotype across environments.

Evolution of reaction norm

Predicting the reaction norm response to selection

From standard quantitative genetics applied to the components of linear reaction norm (Lande, 1976; Gavrillets and Scheiner, 1993; de Jong, 1990), if reaction norm intercept and slope are normally distributed, then the change in the mean reaction norm in response to selection in year j is

$$\Delta \begin{pmatrix} \bar{a}_j \\ \bar{b}_j \end{pmatrix} = G \begin{pmatrix} \beta_{a,j} \\ \beta_{b,j} \end{pmatrix} \quad (7)$$

where $\beta_{a,j}$ and $\beta_{b,j}$ are the selection gradients on reaction norm intercept and slope defined above, and their additive genetic covariance matrix is

$$G = \begin{pmatrix} G_{aa} & G_{ab} \\ G_{ab} & G_{bb} \end{pmatrix} \quad (8)$$

with G_{aa} and G_{bb} the additive genetic variances in intercept and slope respectively, and G_{ab} their additive genetic covariance. Furthermore, combining equations (2), (7) and (8), the response to selection by the reaction norm components in year j can be written as

$$\Delta \begin{pmatrix} \bar{a}_j \\ \bar{b}_j \end{pmatrix} = 0.5 * \beta_{z,j} \begin{pmatrix} G_{aa} & G_{ab} \\ G_{ab} & G_{bb} \end{pmatrix} \begin{pmatrix} 1 \\ E_j \end{pmatrix} = 0.5 * \beta_{z,j} \begin{pmatrix} G_{aa} & G_{ab}E_j \\ G_{ab} & G_{bb}E_j \end{pmatrix}. \quad (9)$$

The predicted response is halved because laying date is a sex limited trait. The last member of this equation can be interpreted as the product of directional selection gradient $\beta_{z,j}$ on the expressed trait in year j , multiplied by the additive genetic covariance between the expressed trait and its reaction norm slope and intercept (elements in the vector), which depend on the environmental cue in that year. The lower row in the vector shows that, for a given strength of phenotypic selection β_z , evolutionary change in plasticity is larger under larger absolute values of $G_{ab} + G_{bb}E$. If reaction norm slope and intercept are genetically uncorrelated ($G_{ab} = 0$), such that phenotypic variance is minimum in environment 0 where reaction norms ‘cross’ (equal to the mean environment if data have been mean-centered, Gavrillets and

Scheiner, 1993; Lande, 2009), then the response to selection of plasticity is larger in more extreme environments. However, this is not true if the slope and intercept are genetically correlated, such that reaction norms cross on average in a non-zero environment.

Estimating the reaction norm G-matrix

Elements of the G-matrix (G) of laying date reaction norm were estimated using a random regression animal model estimating among-individual and among-genotype variation of laying date reaction norm in response to the environmental cue E_j ,

$$LD_{ij} = a + bE_j + \alpha_A A_{ij} + \alpha_{A^2} A_{ij}^2 + (g_i + e_i) + (g_{Ei} + e_{Ei})E_j + Y_j + \epsilon_{ijk} \quad (10)$$

In equation (10), the laying date LD of female i in year j is described by an average population-level mean a and several fixed and random effects. We included age A_{ij} and age-squared A_{ij}^2 fixed effects, because previous analyses indicating that they affect laying date in this population (Bonamour et al. submitted). We modelled plasticity in response to spring temperature using a linear reaction norm. The mean plasticity in the population was estimated through a fixed environmental effect of E_j , and we used random effects to estimate within-population variation in reaction norm intercept and slope. We estimated among-individual (resulting from permanent environment variation, ‘I’) and among-genotype (resulting from additive genetic variation, ‘G’) variation of average laying date via the individual random effect e_i and the genotype random effect g_i respectively. Another set of random effects was used to estimate variation of reaction norm slope (i.e. plasticity): e_{Ei} for the among-individual component of this variation (‘IxE’), and g_{Ei} for the among-genotype component (‘GxE’). Comparing with the simple reaction norm in equation (1), we have $a_i = g_i + e_i$ for the intercept, and $b_i = g_{Ei} + e_{Ei}$ for the slope, or plasticity. Heritability of laying date and laying date plasticity were estimated as $h_{int}^2 = \frac{g_i}{(g_i + e_i + g_{Ei} + e_{Ei} + \epsilon_{ij} + V_F)}$ and $h_{slp}^2 = \frac{g_{Ei}}{(g_i + e_i + g_{Ei} + e_{Ei} + \epsilon_{ij} + V_F)}$ calculated over the posterior distributions of the estimates, where V_F the variance of the values predicted by the model along all possible values for the fixed-effects, as predicated by de Villemereuil et al. (2018).

We also included random effect Y_j for year, in order to control for annual environmental variation in the mean phenotype that could not be attributed to our modelled linear relationship with spring temperature. Laying date variance was heterogeneous with respect to the environmental cue (laying date variance equal to 31.45; 39.40 and 58.46 respectively for the 33% of lowest, 33% median and 33% highest temperature values), so we allowed residuals ϵ_{ijk} to be heterogeneous and uncorrelated, and divided the environment in three categories (by 33% quantiles), to allow correct estimation of the among-individual and -genotype variations (Nicolaus et al., 2013; Ramakers, 2019). Note that changes in the variance of laying date along the environmental

gradient could result from among -individual/-genotype variation in reaction norm slope, which are captured by our random effects, and/or by other factors not included in the analysis (and absorbed by the residuals), such as uncontrolled environmental variation that covaries with the environmental cue, or non-linearity of reaction norms (Ramakers, 2019).

Comparing the predicted versus estimated reaction norm evolution

We then compared the predicted response to selection for both intercept and slope of the laying date reaction norm to the estimated changes in breeding values for these traits. We estimated the annual response to selection observed in the blue tit population for both elevation and slope reaction norm ($\hat{\Delta}_{\bar{g}_a}$ and $\hat{\Delta}_{\bar{g}_b}$) as the difference between the mean breeding values of local recruits in year t and the mean breeding values of all adult individuals breeding in year $t - 1$ (corresponding to the parental cohort). The breeding values were estimated from the BLUPS of the random regression animal model (equation 10). We then compared these estimated $\hat{\Delta}_{\bar{g}_a}$ and $\hat{\Delta}_{\bar{g}_b}$ to the response to selection predicted from equation 9 ($\Delta_{\bar{a}}$ and $\Delta_{\bar{b}}$).

Cost of plasticity

We were also interested in finding out whether phenotypic plasticity is costly *per se*, beyond its influence on selection on the expressed laying date across environments. We build on the model suggested by (Scheiner and Berrigan, 1998; Van Buskirk and Steiner, 2009), but allowing to assess the costs across rather than within environments. For a linear reaction norm, the cost of plasticity can be estimated in a model where (annual) relative fitness is determined by the trait, the environmental cue, their interaction – measuring selection on plasticity via its effect on the expressed trait (equation 3-4) –, and reaction norm slope – measuring the cost of plasticity. Hence, we used the following linear mixed model for the relative fitness W of female i in the year j

$$W_{ij} = \mu + \beta_{E,0}LD_{ij} + \alpha_E E_j + \beta_E(LD_{ij}E_j) + c(b_i) + \alpha_A A_{ij} + \alpha_{A^2} A_{ij}^2 + I_i + Y_j + \epsilon_{ijk} \quad (11)$$

The selection components of this model include: i) an intercept $\beta_{E,0}$ for selection on the trait in the average environment, ii) an interaction between laying date and temperature, with coefficient β_E , capturing selection on plasticity via its effect on the expressed laying date (as in equation 3-4); and iii) an effect the estimated slope reaction norm b_i , for which the coefficient c is the cost of plasticity. Other effects as in equation (4). The estimated individual phenotypic slope of laying date reaction norm (b_i) are calculated as the sum of the individual (e_{Ei}) and genotype (g_{Ei}) BLUPS of reaction norm slope with temperature and the population-level slope (b), estimated from the random regression animal models (equation 10). In order to take into account error

estimation of BLUPS, we implemented equation (11) for one hundred random selected iterations of the equation (10), and we then estimated the posterior distribution of the estimated cost of plasticity (as recommended in Hadfield et al., 2010).

All models are run using a Bayesian framework based on a Markov Chain Monte Carlo algorithm using the software R (package MCMCglmm, Hadfield, 2010, version 3.4.4, R Core Team, 2018) and error was represented by the 95% credible intervals (see supplementary material 2 for details about prior implementation and diagnostic processes).

3. Results

3.1 Fluctuating environment

Among-year variation of the ‘bird – prey time lag’ is observed in the studied population (with variance of the ‘bird – prey time lag’ around 62 with mean of c. -26 days) but no temporal trend is detected (no correlation between the ‘bird – prey time lag’ and year: slope = 0.02, SE = 0.12, t-value = 0.17, p-value = 0.86, $r^2 = 0.03$, figure 1b). There is also among-year variation of cue temperature and a strong climate warming (change of +0.65 °C/decade during this time window, SE = 0.008, t-value = 7.8, p-value = 1.41e-09, $r^2 = 0.60$), figure 1b and see Bonamour et al., 2019).

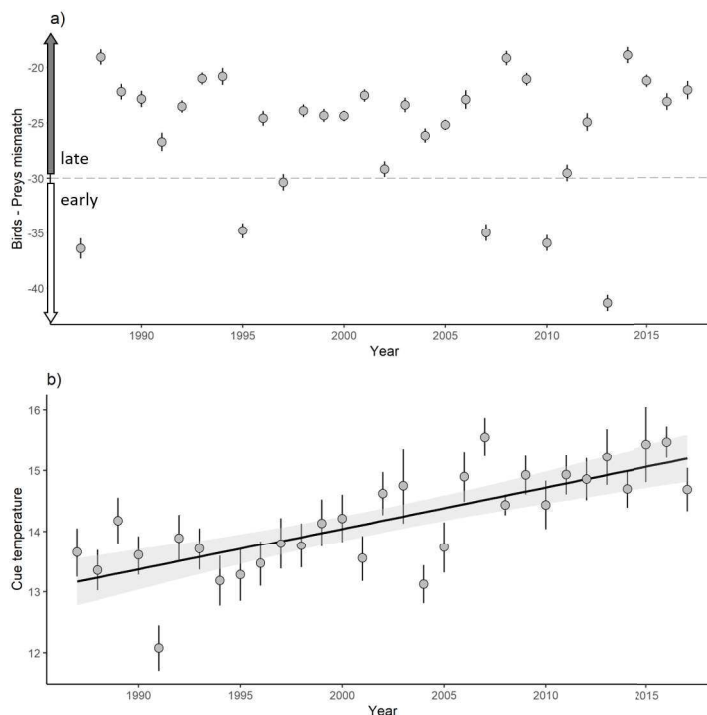


Figure 1: Fluctuating environment. a) The ‘bird – prey time lag’ (in days \pm standard error) variation through years. Grey dashed lines correspond to the optimal expected ‘bird – prey time lag’ of 30 days; b) Cue temperature warming through years (in °C \pm standard error).

3.2 Natural selection on reaction norm

Annual selection on the expressed laying date

There is overall directional fecundity selection for earlier laying date over the monitored period (selection gradient: -0.01 [-0.02; -0.01], supplementary material 3). When estimated annually, selection gradient on laying date receive strong statistical support in eight years (i.e. the 95% credible intervals exclude zero, supplementary materials 4 and 5). Estimated annual selection gradients are associated with large credible intervals (supplementary material 5), however, models estimating average selection on laying date and its among-years fluctuations including year as a random effect allows better estimation of the variation of selection because of higher statistical power (Chevin et al., 2015). Note that using this method, de Villemereuil and collaborators (in prep.) show that selection on laying date fluctuates among-years.

Annual selection on slope

Estimation of selection on laying date plasticity, obtained by combining annual selection gradients on laying date with annual temperature cues, exclude zero in the same eight years as selection on the expressed trait (electronic supplementary materials 4 and 5). The median selection on laying date plasticity with respect to spring temperature over the studied period, estimated using equation 3, is 0.003 [0.000; 0.006]. Note however that the 95% credible interval marginally include zero (table 1).

Partitioning selection

To investigate whether the environmental cue driving plasticity is a good predictor of the selection remaining after taking into account selection on the trait, we estimated the change in selection on laying date in response to the temperature cue (equations 3-4). Results show a positive interaction between laying date and temperature on annual reproductive success (but note that the credible interval marginally include zero, table 1). As directional selection is overall negative (towards earlier laying date), this indicates that selection favours less plasticity over our study period because of increasing temperature cue (equation 3). In addition, we found a negative interaction between laying date and the ‘bird – prey time lag’ on annual reproductive success, meaning that directional selection on laying date increases with larger ‘bird – prey time lag’, that is when birds lay too late comparing to the caterpillar’s phenology (table 2).

Table 1 Partitioning selection on laying date. Results of the model 4. In bold, fixed effect estimate with 95% credible interval excluding zero. In italic, fixed effect estimate with 95% credible interval marginally including zero.

Fixed effects	
Intercept (μ)	1.84 [1.37 ; 2.67]
LD ($\beta_{E,0}$)	-0.01 [-0.01 ; -0.005]
Cue (α_E)	-0.61 [-1.14 ; 0.02]
Age (α_A)	0.17 [0.11 ; 0.24]
Age² (α_A^2)	-0.03 [-0.04 ; -0.02]
<i>LD : Cue</i> (β_E)	<i>0.01</i> [<i>-0.00</i> ; <i>0.01</i>]
Random effects	
Year (Y_j)	0.004 [0.00; 0.01]
Ind (I_i)	0.03 [0.01; 0.04]
Residuals	
Units (ϵ_{ij})	0.22 [0.20; 0.24]

Table 2 The ‘bird – prey time lag’ as a cause of selection. Results of the model 6. In bold, fixed effect estimate with 95% credible interval excluding zero.

Fixed effects	
Intercept (μ)	1.29 [0.62 ; 2.09]
LD ($\beta_{U,0}$)	-0.003 [-0.01; 0.002]
Time-lag (α_U)	0.08 [0.03 ; 0.13]
Age (α_A)	0.16 [0.10 ; 0.22]
Age² (α_A^2)	-0.03 [-0.04 ; -0.02]
LD : Time-lag (β_U)	-0.0007 [-0.001 ; -0.0003]
Random effects	
Year (Y_j)	0.004 [0.002; 0.01]
Ind (I_i)	0.02 [0.01; 0.04]
Residuals	
Units (ϵ_{ij})	0.22 [0.20; 0.24]

3.3 Reaction norm variability and heritability

Laying date varies with spring temperature, with an average population slope of -4.19 day/ $^{\circ}\text{C}$ (table 3, figure 2a). Individuals differ in their laying date intercept and plasticity (respectively ‘I’ and ‘IxE’), with a weak positive correlation between phenotypic reaction norm intercept and slope (see supplementary material 6). In addition we found additive genetic variance of laying date intercept (‘G’) and plasticity (‘GxE’, table 3, figure 2c), with heritabilities of 0.13 [0.05; 0.26] and 0.11 [0.03; 0.19], respectively. Phenotypic plasticity is therefore heritable in this population, and may thus evolve in response to selection. In equation (10) we fixed covariance of individual and genetic intercept and slope reaction norm to zero because of low robustness for covariance estimations (diagnostics not shown here). Note however that variance components were equivalent in models estimating individual and animal intercept – slope covariances (see supplementary material 6 for other tested models).

Table 3 Among -individual and -genotype variation of laying date plasticity with temperature. Results of the model 10. In bold, estimates with 95%CI excluding zero. See figure 2 for graphical representations.

Fixed effects	
Intercept (<i>a</i>)	133.53 [132.26; 135.04]
Temperature (<i>b</i>)	-4.19 [-5.49; -2.50]
Age (α_A)	-3.41 [-3.75; -3.07]
Age² (α_A^2)	0.41 [0.36; 0.46]
Random effects	
Year	14.29 [8.75; 23.04]
I (e_i)	10.38 [4.42; 15.00]
IxE (e_{Ei})	10.89 [3.46; 15.36]
G (g_i)	7.62 [2.70; 13.42]
GxE (g_{Ei})	4.34 [1.38; 12.18]
Residuals	
Residual 1	6.83 [6.18; 7.42]
Residual 2	4.03 [3.38; 4.72]
Residual 3	8.00 [6.90; 9.15]

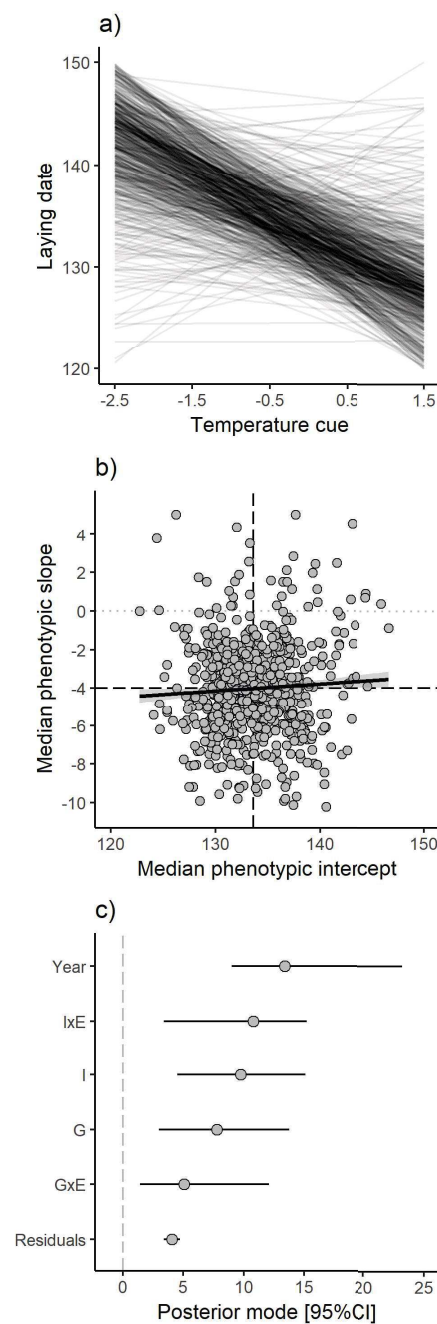


Figure 2: Among-individual laying date reaction norm variation. a) Estimated individual phenotypic reaction norm of laying date with temperature from equation 10; b) Covariance between phenotypic values of reaction norm intercept and slope (slight positive correlation between phenotypic intercept and slope reaction norm: slope = 0.10, SE = 0.05, t-value = 2.11, p-value = 0.04); c) Variance component estimates from model 10 (posterior mode \pm 95% confidence intervals, the three residuals estimates are given in table 3). The intercept among-individual variation ‘I’ explain 20% of the total population-level phenotypic variance of laying date (equal to 51.55 over the studied period 1979 – 2017), while the slope among-individual ‘IxE’, the intercept ‘G’ and slope ‘GxE’ among-genotype variations explain 22%, 15% and 9% respectively (assuming a Gaussian distribution of the temperature cue, Morrissey and Liefting, 2016).

3.4 Reaction norm evolution

Because we found evidence for additive genetic variance in both reaction norm slope and intercept, these traits may evolve in response to selection on laying date reaction norm. Annual predicted responses to selection exclude zero in their 95% credible intervals for the same eight years where the selection gradients were significant (figure 3, supplementary material 4).

The annual average estimated evolution of laying date breeding value correlates with the posterior mode of the predicted response to selection (estimated $\hat{\Delta}_{\bar{g}_a}$ – predicted $\hat{\Delta}_{\bar{g}_a}$: intercept = 0.29, SE = 0.18, t-value = 1.66, p-value = 0.11; slope = 0.93, SE = 0.24, t-value = 3.85, p-value = 0.0005, $r^2 = 0.28$, figure 3b). On the contrary, the correlation is positive but not significant for the evolution of the slope (estimated $\hat{\Delta}_{\bar{g}_b}$ – predicted $\hat{\Delta}_{\bar{g}_b}$: intercept = -0.10, SE = 0.07, t-value = -1.31, p-value = 0.20; slope = 0.14, SE = 0.16, t-value = 0.87, p-value = 0.39, $r^2 = 0.01$, figure 3d).

3.5 Is there a cost of plasticity?

We estimated a marginally significant variation of relative annual reproductive success according to the slope reaction norm (equation 11) of -0.004 [-0.01; 0.001], which is comparable to the average strength of selection on plasticity via its effect on the expressed laying date over the study period.

4. Discussion

We found temporally variable natural selection on laying date reaction norm coupled with additive genetic variation of both intercept and slope of reaction norm in a free-living blue tit population. Overall, selection analyses seem to indicate that the blue tit phenology is on average too late, especially in the coldest years. Our results seem to indicate that the mean breeding value fluctuates over time for both the intercept and slope of the reaction norm, and that these changes are correlated with the responses to selection predicted based on the estimated selection gradients and additive genetic (co)variances, for reaction norm intercept but not for its slope (i.e. plasticity). However, these results need to be taken with caution, as we were unable yet to fully account for all sources of uncertainty when predicting responses to selection, so more analyses are clearly needed to reach robust conclusions on responses to selection by the reaction norm.

Laying date has previously been shown to be under negative selection in many wild bird populations (e.g. Sheldon et al., 2003; Gienapp et al., 2006; Brommer and Rattiste, 2008; Charmantier et al., 2008; Sirkiä et al., 2018, and our results show similar strength as in other blue tit populations, Porlier et al., 2012; Marrot et al., 2018). Annual selection on laying date in our study population is correlated to the ‘bird – prey time-lag’, but it seems that most of the variation in selection is not explained by the change in caterpillar phenology. Other environmental driver(s) of selection on laying date are likely to exist in

the forest, such as the presence of predators of birds in the breeding seasons, spring temperature during the rearing period of chicks and/or the temperature extreme events during the year, as found by Marrot and collaborators in another blue tit population (Marrot et al., 2017, 2018).

There are fewer studies estimating natural selection on plasticity in natural conditions (e.g. Brommer et al., 2005, 2012; Nussey et al., 2005). Annual estimates of selection on reaction norm are even scarcer in natural population. In fact we are only aware of one study that estimated annual selection gradients on plasticity in wild population (Great tits, Ramakers et al., 2018). Using, as we did, selection on the expressed trait across environments to estimate selection on slope (see van Tienderen and Koelewijn, 1994 for early developments of this method), this study found on average no selection on reaction norm slope (Ramakers et al., 2018). Similarly, in a meta-analysis of selection on thermal plasticity in the few studied populations of plants and birds, Arnold and collaborators (Arnold et al., 2019) found no evidence for overall significant natural selection, despite individual cases of selection for more and less plasticity in their datasets.

Among the few studies estimating directional selection gradients on plasticity reaction norm (both intercept and slope), selection is commonly estimated using a multivariate model, aiming to assess the covariance between lifetime reproductive success and the trait intercept and slope (multivariate random regression, e.g. Brommer et al., 2012; Ramakers et al., 2018; Arnold et al., 2019). Such models partition selection on the trait from selection on the plasticity of the trait, but in fact the estimated selection on plasticity includes both the influence of plasticity on the expressed trait and any putative costs (or benefits) of plasticity *per se*. We propose disentangling these two components using a model where fitness depends on both the expressed trait value in each environment (rather than the reaction norm intercept) and plasticity (equation 11). Contrary to expectation (see e.g. Dewitt et al., 1998), it seems that laying date plasticity is not costly in our blue tit population. This may be explained by that fact that females in better body conditions may display stronger plasticity and higher reproductive success, but more studies are needed to conclude. In addition, deriving selection on plasticity from selection on the expressed trait across environments, as already partly implemented by previous authors (van Tienderen and Koelewijn, 1994; Ramakers et al., 2018), allows estimating annual selection gradients and predicted responses to selection of the reaction norm components, opening the door for the analysis of evolution of reaction norms under fluctuating selection in wild populations.

We found a surprisingly high heritability of laying date plasticity in our studied population (figure 2, table 3), almost as high as the heritability of laying date itself. This contrasts with the few available studies estimating heritability of plasticity in wild populations, which often detected no (or very small) additive variance of the slope reaction norm (e.g. Brommer et al., 2005, 2008; Husby et al., 2010; Ramakers, 2019).

We predicted a significant response to selection of reaction norm slope and intercept in eight years out of forty, but preliminary analyses do not seem to

detect as much change in the breeding values of the population. The more probable explanation is lack of statistical power as the expected changes are about -0.4 days on average (ranging from -3.4 to 1.0 days) and -0.05 days/°C on average (ranging from -1.44 to 1.31 days/°C) per generation. Another potential reason for the difference between predicted and observed responses is that evolution predicted via the breeder's equation may lead to over-estimation of predicted response in wild populations (Morrissey et al., 2010) for example because of a discrepancy between the trait – fitness relationship at the phenotypic and genetic levels (Pujol et al., 2018). One way to resolve this issue could be to implement a model to estimate the response to selection of the reaction norm directly estimating genetic covariance of fitness and reaction norm slope, using a ‘bivariate Robertson-Price identity’ (as in the ‘bivariate model’ of e.g. Brommer et al., 2012; Arnold et al., 2019 but directly at the genetic levels, see introduction). Finally, we chose to predict response to selection of laying date reaction norm using the genetic covariance matrix of the intercept and slope with covariance fixed to zero, for the sake of parsimony, because the covariances were not really well estimated by our models. However, the genetic covariance between intercept and slope reaction norm is likely negative, potentially leading to evolutionary constraints.

Overall, our results suggest that selection on plasticity in our blue tit population is weak on average but fluctuates among years, although we detect ongoing climate warming. Phenotypic changes mediated by phenotypic plasticity are among the main responses to climate change, and environmental change may modify the adaptiveness of reaction norm in wild populations. For now at least, the plasticity of the blue tit in the studied population may be sufficient to allow birds coping with the earlier phenology of their preys due to climate change. However, although the ‘bird – prey time-lag’ is fluctuating but stable over time, the caterpillars are more plastic than the birds (Bonamour et al., 2019), such that the rate of plastic phenological change should be faster in caterpillars than in birds in the future, and maybe, increase selection on bird plasticity.

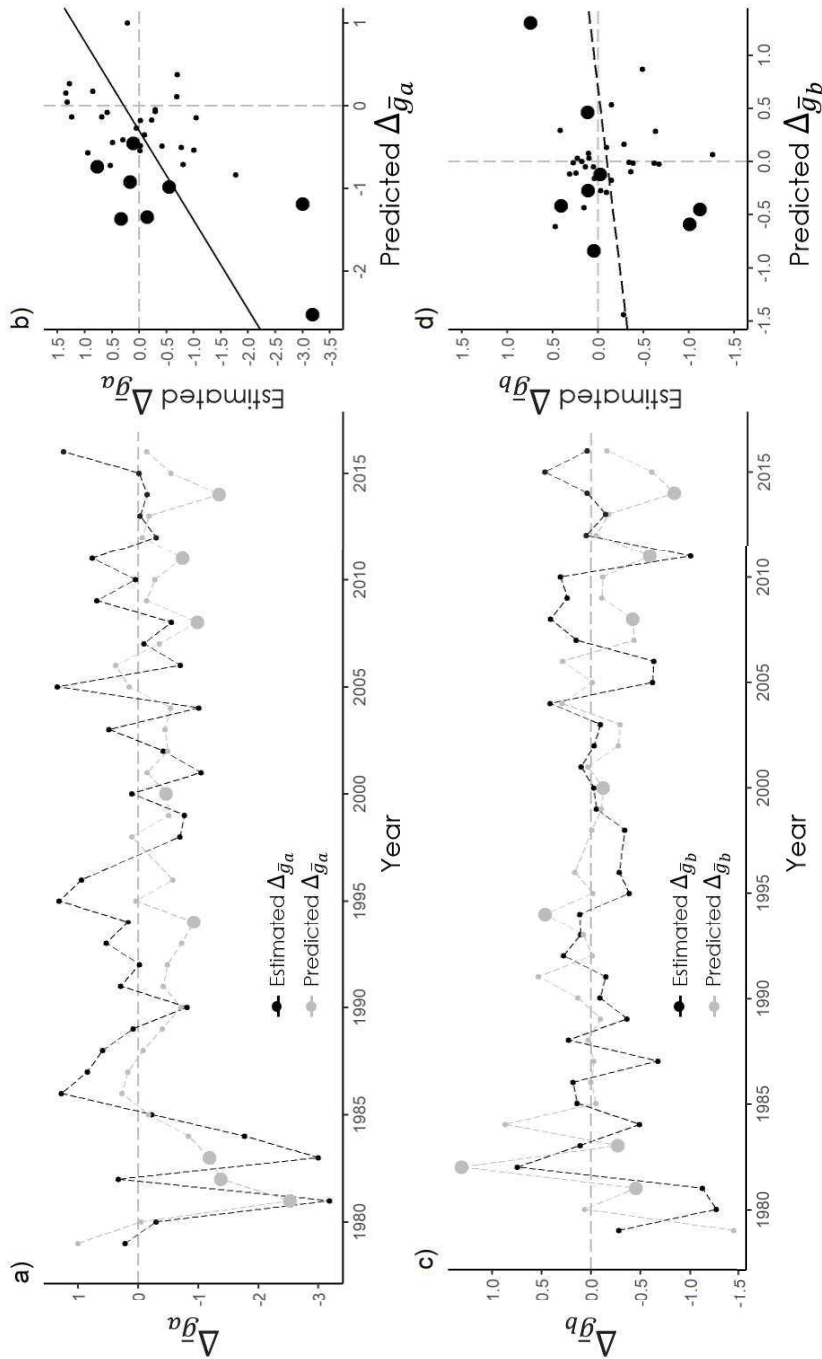


Figure 3: Change in laying date reaction norm. Genetic change in intercept a) predicted (in black) and estimated (in grey) change through time, b) estimated against predicted genetic change; Genetic change in slope c) predicted (in black) and estimated (in grey) change through time, d) estimated against predicted genetic change. For the predicted changes (in grey), small dots are estimates with 95% credible interval including zero, large dots are estimates with 95% credible interval excluding zero. We do not represent credible intervals for readability, see supplementary material 4).

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Ethics

All experimental protocols described here were approved by the ethics committee for animal experimentation of Languedoc Roussillon (305-CEEA-LR-12066 approved in 2012) as well as by Regional Institutions (bylaw issued by the Prefecture of Haute-Corse on 17/04/2018 n° 2B-2018-04-17-001). Captures were performed under a personal ringing permit delivered by the CRBPO (Centre de Recherches par le Bagueage des Populations d'Oiseaux) to Anne Charmantier (ringing permit number 1907).

Data accessibility

Data will be available in HAL repository (hal.archives-ouvertes.fr).

Author's contributions

L.-M.C., S.B., C.T. and A.C. designed the research. A.C., C.T., O.B. and S.B. (and many other contributors) collected field data. S.B. conducted analyses. S.B., O.B., C.T., L.-M.C., A.C., D.R. wrote the paper.

Competing interests

We declare we have no competing interests.

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Supplementary materials

Supplementary material 1

Samples sizes.

The overall data set include 1696 laying date observations for 851 identified females. Annual reproductive success is known for 1653 laying date attempts.

Year	Number of laying date observation	Number of reproductive success observation
1979	5	5
1980	8	8
1981	10	10
1982	15	15
1983	19	19
1984	16	16
1985	24	24
1986	30	30
1987	43	43
1988	54	54
1989	46	46
1990	50	50
1991	48	48
1992	58	58
1993	66	66
1994	40	40
1995	52	52
1996	47	47
1997	34	34
1998	35	34
1999	40	40
2000	67	67
2001	57	57
2002	42	42
2003	41	40
2004	39	39
2005	53	53
2006	56	53
2007	72	72
2008	54	54
2009	48	48
2010	58	40
2011	60	60
2012	46	35
2013	52	52
2014	46	45
2015	49	47
2016	58	51
2017	58	56

Supplementary material 2

Detailed of Bayesian implementation in MCMCglmm package.

All models are run using a Bayesian framework based on a Markov Chain Monte Carlo algorithm using the software R (package MCMCglmm, Hadfield, 2010, version 3.4.4, R Core Team, 2018) and error was represented by the 95% credible intervals. Fixed effects were modelled using a Gaussian non-informative priors, with mean zero and variance 10^8 . Random effects and residual variances were modelled using an inverse-Wishart $w^{-1}(\Psi, \nu)$ priors, see below for specification for each models. For all models, the maximum autocorrelation between estimates is < 0.05 for fixed and random effects.

Equation 2: Annual selection gradient. ($\beta_{z,j}$)

Number of iteration: 10001000

Burn-in period: 1000

Thin: 1000

Random and residual effect priors: No random effect. Residual effect: $\Psi =$ the phenotypic variance of relative annual reproductive success $\nu = 1$.

Equation 4 and 6: Partitioning selection on reaction norm and the ‘bird – prey time-lag’.

Number of iteration: 10010000

Burn-in period: 100000

Thin: 5000

Random and residual effects prior: $\Psi =$ the phenotypic variance of relative annual reproductive success divided by the number of estimated variance components $\nu = 1$.

Equation 10: Random regression animal model.

Number of iteration: 10010000

Burn-in period: 10000

Thin: 1000

Random and residual effect priors: $\Psi =$ the phenotypic variance of laying date divided by the number of estimated variance components. Convergence of MCMC chains was assessed by comparing several chains run under different prior values of $\nu = 1, 0.2$ and 0.02 (ν is the degree of belief in the prior), using the Gelman and Rubin’s convergence diagnostic (Gelman and Rubin, 1992; Brooks and Gelman, 1998). Models converged well, with an average Gelman’s diagnostic $R^2 = 1.00$ for fixed and random effects.

Equation 11: Cost of plasticity.

Number of iteration: 10010000

Burn-in period: 100000

Thin: 5000

Random and residual effects prior: $\Psi =$ the phenotypic variance of laying date divided by the number of estimated variance components. Convergence

of MCMC chains was assessed by comparing several chains run under different prior values of $\nu = 1, 0.2$ and 0.02 (ν is the degree of belief in the prior), using the Gelman and Rubin's convergence diagnostic (Gelman and Rubin, 1992; Brooks and Gelman, 1998). Models converged well, with an average Gelman's diagnostic $R^2 = 1.10$ for fixed and random effects.

Supplementary material 3

Global selection on laying date.

We estimated selection on laying date over the studied period (1979 – 2017) as:

$$ARS_{ij} = \mu + \alpha_{LD}LD_{ij} + I_i + Y_j + e_{ij} \quad (\text{S3})$$

Where the relative annual reproductive success ARS (estimated as the number of fledgling of the first clutch of the year) of the female i in the year j varies according to the average population level μ and the laying date LD_{ij} . We added an individual random effect I_i to taking into account non-independence of phenotypic observation on same individuals and a year random effect Y_j in order to take into account environmental variation not included in the model. e_{ij} are residuals. The model S3 was implemented in a Bayesian framework using a Markov Chain Monte Carlo estimation algorithm using the software R (package MCMCglmm, Hadfield, 2010, version 3.4.4, R Core Team, 2018). We used Gaussian non-informative priors, with mean zero and variance 10^8 for the fixed effects, while random effects and residual variance were models using a little informative prior, corresponding to an inverse-Wishart $w^{-1}(\Psi, \nu)$ distribution with Ψ equal to the phenotypic variance of laying date divided by the number of estimated variance components. Convergence of MCMC chains was assessed by comparing several chains run under different prior value of $\nu = 1, 0.2$ or 0.02 (ν is the degree of belief in the prior), using the Gelman and Rubin's convergence diagnostic (Gelman and Rubin, 1992; Brooks and Gelman, 1998). Models converged well, with an average Gelman's diagnostic $\hat{R} = 1.00$ for fixed and random effects. Model were run for 5,030,000 iterations, including a burn-in period of 30,000 iterations sampled every 1000 iteration. The maximum autocorrelation between estimates is < 0.05 for fixed and random effects.

Results of the equation S3:

We present posterior modes and their 95% credible interval of the model with $\nu = 1$.

Fixed effects	
Intercept (μ)	2.77 [2.28, 3.38]
LD (α_{LD})	-0.01 [-0.02, -0.01]
Random effects	
Year ($year_j$)	0.02 [0.01, 0.04]
Individual (ind_i)	0.02 [0.01, 0.04]
Residuals	
Units (e_{ij})	0.20 [0.18, 0.21]

Supplementary material 4

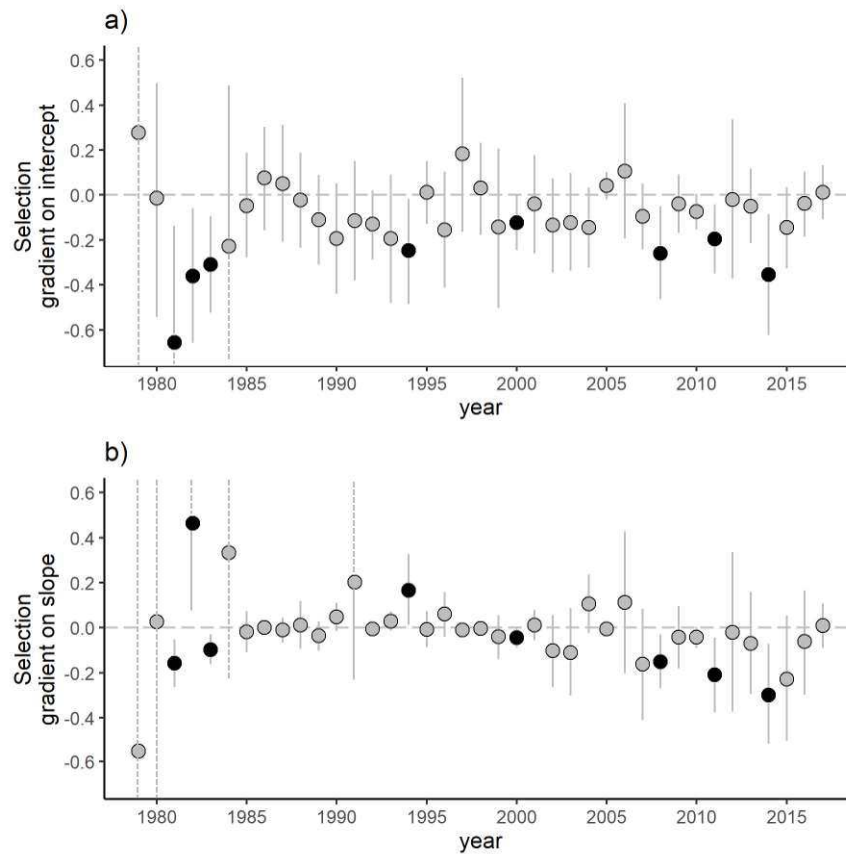
Annual selection gradients and expected response to selection of laying date reaction norm intercept and slope.

Year	Mean-centered temperature (°C)	Selection gradients		Expected response to selection	
		Intercept	Slope	Intercept	Slope
1976	-2.21	0.01 [-0.13; 0.15]	-0.01 [-0.32; 0.29]	0.02 [-0.58; 0.64]	-0.03 [-1.20; 1.07]
1977	-0.45	-0.87 [-1.39; -0.35]	0.39 [0.16; 0.63]	-3.37 [-7.48; -0.96]	1.13 [0.28; 3.01]
1978	-1.62	0.17 [-0.66; 0.97]	-0.27 [-1.57; 1.06]	0.59 [-2.82; 4.65]	-0.70 [-6.14; 3.85]
1979	-2	0.28 [-0.82; 1.42]	-0.56 [-2.84; 1.64]	1.00 [-3.57; 6.57]	-1.44 [-11.14; 5.70]
1980	-1.99	-0.01 [-0.54; 0.50]	0.03 [-0.99; 1.08]	-0.05 [-2.39; 2.25]	0.06 [-3.75; 3.97]
1981	0.24	-0.65 [-1.09; -0.22]	-0.16 [-0.26; -0.05]	-2.53 [-5.84; -0.65]	-0.45 [-1.25; -0.10]
1982	-1.29	-0.36 [-0.66; -0.06]	0.46 [0.08; 0.85]	-1.37 [-3.40; -0.19]	1.31 [0.17; 3.81]
1983	0.31	-0.31 [-0.52; -0.10]	-0.10 [-0.16; -0.03]	-1.19 [-2.69; -0.28]	-0.27 [-0.77; -0.06]
1984	-1.46	-0.23 [-0.72; 0.26]	0.33 [-0.38; 1.05]	-0.84 [-3.46; 1.14]	0.87 [-1.20; 4.20]
1985	0.39	-0.05 [-0.28; 0.19]	-0.02 [-0.11; 0.07]	-0.17 [-1.29; 0.81]	-0.05 [-0.42; 0.26]
1986	0.01	0.08 [-0.16; 0.30]	0.00 [0.00; 0.00]	0.27 [-0.70; 1.42]	0.00 [0.00; 0.01]
1987	-0.21	0.05 [-0.21; 0.31]	-0.01 [-0.06; 0.04]	0.18 [-0.92; 1.45]	-0.03 [-0.25; 0.16]
1988	-0.5	-0.02 [-0.23; 0.19]	0.01 [-0.09; 0.12]	-0.08 [-1.10; 0.85]	0.03 [-0.36; 0.47]
1989	0.32	-0.11 [-0.31; 0.09]	-0.04 [-0.10; 0.03]	-0.41 [-1.52; 0.36]	-0.10 [-0.42; 0.09]
1990	-0.25	-0.19 [-0.44; 0.05]	0.05 [-0.01; 0.11]	-0.71 [-2.16; 0.20]	0.13 [-0.04; 0.48]
1991	-1.78	-0.11 [-0.38; 0.15]	0.20 [-0.27; 0.68]	-0.42 [-1.81; 0.63]	0.53 [-0.90; 2.71]
1992	0.04	-0.13 [-0.29; 0.02]	0.00 [-0.01; 0.00]	-0.49 [-1.44; 0.08]	-0.01 [0.00; -0.05]
1993	-0.15	-0.19 [-0.48; 0.09]	0.03 [-0.01; 0.07]	-0.72 [-2.35; 0.35]	0.08 [0.30; -0.04]
1994	-0.67	-0.25 [-0.48; -0.02]	0.17 [0.01; 0.33]	-0.93 [-2.45; -0.07]	0.46 [1.48; 0.03]
1995	-0.58	0.01 [-0.13; 0.15]	-0.01 [-0.09; 0.07]	0.04 [-0.59; 0.69]	-0.02 [0.28; -0.34]
1996	-0.39	-0.16 [-0.41; 0.10]	0.06 [-0.04; 0.16]	-0.57 [-1.99; 0.41]	0.16 [0.68; -0.12]
1997	-0.06	0.18 [-0.16; 0.52]	-0.01 [-0.03; 0.01]	0.67 [-0.65; 2.53]	-0.03 [0.03; -0.13]
1998	-0.09	0.03 [-0.18; 0.23]	0.00 [-0.02; 0.02]	0.11 [-0.80; 1.05]	-0.01 [0.06; -0.08]
1999	0.28	-0.14 [-0.50; 0.21]	-0.04 [-0.14; 0.06]	-0.50 [-2.36; 0.88]	-0.10 [0.19; -0.57]
2000	0.36	-0.12 [-0.25; 0.00]	-0.04 [-0.09; 0.00]	-0.46 [-1.25; 0.00]	-0.12 [0.00; -0.39]
2001	-0.3	-0.04 [-0.26; 0.18]	0.01 [-0.05; 0.08]	-0.15 [-1.21; 0.77]	0.03 [0.31; -0.19]
2002	0.76	-0.13 [-0.35; 0.07]	-0.10 [-0.26; 0.06]	-0.49 [-1.70; 0.29]	-0.27 [0.17; -1.15]
2003	0.89	-0.12 [-0.34; 0.10]	-0.11 [-0.30; 0.09]	-0.45 [-1.64; 0.40]	-0.29 [0.27; -1.25]
2004	-0.73	-0.15 [-0.32; 0.03]	0.11 [-0.02; 0.24]	-0.54 [-1.63; 0.13]	0.29 [1.02; -0.07]
2005	-0.12	0.04 [-0.02; 0.10]	-0.01 [-0.01; 0.00]	0.15 [-0.08; 0.50]	-0.01 [0.01; -0.06]
2006	1.04	0.11 [-0.19; 0.41]	0.11 [-0.20; 0.42]	0.38 [-0.85; 1.92]	0.28 [1.75; -0.68]
2007	1.7	-0.10 [-0.24; 0.05]	-0.16 [-0.41; 0.08]	-0.35 [-1.20; 0.19]	-0.44 [0.25; -1.74]
2008	0.58	-0.26 [-0.46; -0.05]	-0.15 [-0.27; -0.03]	-0.99 [-2.47; -0.16]	-0.42 [-0.06; -1.26]
2009	1.07	-0.04 [-0.17; 0.09]	-0.04 [-0.18; 0.10]	-0.14 [-0.80; 0.40]	-0.11 [0.35; -0.72]
2010	0.58	-0.07 [-0.15; 0.00]	-0.04 [-0.09; 0.00]	-0.27 [-0.77; 0.02]	-0.12 [0.01; -0.40]
2011	1.07	-0.20 [-0.35; -0.04]	-0.21 [-0.38; -0.04]	-0.74 [-1.82; -0.13]	-0.59 [-0.10; -1.75]
2012	1	-0.02 [-0.37; 0.34]	-0.02 [-0.37; 0.34]	-0.07 [-1.70; 1.51]	-0.05 [1.28; -1.40]
2013	1.37	-0.05 [-0.21; 0.12]	-0.07 [-0.29; 0.16]	-0.18 [-1.04; 0.49]	-0.18 [0.56; -1.20]
2014	0.84	-0.35 [-0.62; -0.08]	-0.30 [-0.52; -0.07]	-1.35 [-3.24; -0.28]	-0.84 [-0.15; -2.44]
2015	1.56	-0.15 [-0.33; 0.03]	-0.23 [-0.51; 0.05]	-0.54 [-1.62; 0.12]	-0.61 [0.14; -2.24]
2016	1.6	-0.04 [-0.18; 0.10]	-0.06 [-0.30; 0.16]	-0.14 [-0.87; 0.45]	-0.16 [-1.19; 0.58]
2017	0.82	0.01 [-0.11; 0.13]	0.01 [-0.09; 0.11]	0.04 [-0.48; 0.61]	0.03 [-0.33; 0.42]

Supplementary material 5

Selection on both intercept and slope reaction norm.

Directional selection gradient (posterior mode \pm 95% credible intervals) on reaction norm a) intercept and b) slope. In black, gradients excluding zero in the 95% credible intervals; in grey, gradient including zero in the 95% credible interval.



Supplementary material 6

Tested models and their variance components.

Heterogeneous residuals. Heritabilities are estimated on the 2nd residual and taking into account fixed effect according to de Villemerueil et al., 2018.

Model	Fixed effects	Random effects	Year	ID (I)	Animal (G)	I*E	G*E	Cov (I, I*E)	Cov (G, G*E)	Units	h ² (int)	h ² (slope)
a	LD ~1	ID + Animal	-	3.90	29.69	-	-	-	-	27.44 ; 17.16 ; 31.14	0.56	-
b	LD ~1	year + ID + Animal	28.37	8.20	9.39	-	-	-	-	11.73 ; 6.94 ; 13.65	0.17	-
c	LD ~1	year + ID + Animal + ID E	30.65	8.42	8.52	15.86	-	2.27	-	8.26 ; 4.99 ; 9.58	0.14	-
d	LD ~1	year + ID + Animal + ID E + Animal E	27.34	8.68	8.72	11.63	4.85	4.65	-1.41	8.31 ; 4.83 ; 9.44	0.13	0.09
e	LD ~age	year + ID + Animal + ID E + Animal E	27.48	8.67	8.21	10.73	6.10	2.53	-1.82	7.90 ; 4.70 ; 8.87	0.13	0.10
f	LD ~age + age ²	year + ID + Animal + ID E + Animal E	25.97	8.32	7.74	9.48	6.27	2.09	-1.27	6.74 ; 3.87 ; 7.87	0.13	0.10
c	LD ~age + age ² + E	year + ID + Animal + ID E + Animal E	13.63	8.68	8.11	8.14	6.00	2.17	-2.60	6.67 ; 4.02 ; 8.00	0.13	0.11
1	LD ~age + age ² + E	year + ID + Animal + ID E + Animal E	14.29	10.38	7.62	10.87	4.34	-	-	6.83 ; 4.03 ; 8.00	0.12	0.10



A punky male blue tit.

(Credits: Chrisitophe de Franceschi)

General Discussion

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The study of phenotypic plasticity has received growing interest in recent decades, since this mechanism is a key driver of phenotypic change (Pigliucci, 2001; DeWitt and Scheiner, 2004). Recently, an increasing number of long-term individual monitorings in natural populations has fuelled the understanding that plastic change is one of the main response to the current global change in the wild (Matesanz et al., 2010; Charmantier et al., 2014; Gienapp and Brommer, 2014; Merilä and Hendry, 2014; Scheffers et al., 2016). With this thesis, combining empirical and theoretical approaches and with the Mediterranean blue tits as a case study, we contribute to the knowledge currently accumulating on the processes involved in phenotypic plasticity and its role in adaptation in nature.

1. What have we learnt?

In **chapter one**, *manuscript A* shows how the current climate change can influence adaptive plasticity, sometimes destabilizing the relationship between the environmental cue(s) inducing a plastic response, the phenotype and the selective environment. Such disruption can alter the relative importance of the benefits and the costs of being plastic, and hence influence the evolutionary trajectory of plasticity. We also show how studying plasticity across several populations of the same species and their preys can enlighten us about how diverse environmental cue windows may be impacted differently by climate change, which may lead to lower cue reliabilities, potentially explaining mismatches between trophic levels, e.g. the blue tits and caterpillars.

Chapter two describes how laying date and its plasticity can vary within-individual lifetimes, and how such age-specific trajectory of plasticity can be related to fitness of female blue tits (*manuscript B*). *Manuscript C* investigates the influence of the experienced environmental quality during lifetime on reproductive performances such as annual reproductive success and laying date. Hence, *manuscript B* focuses on ‘senescence of plasticity’ while *manuscript C* identifies a kind of ‘plasticity of senescence’. This latter chapter illustrates how the plasticity of a phenological trait may be involved in a complex within-individual pattern, plausibly leading to a nontrivial within-individual variation of the adaptiveness the laying date plasticity.

Chapter three focuses on natural selection on both laying date and its plasticity in response to spring temperature, offering in particular some methodological advances to improve our selection measures. *Manuscript D* highlights the importance of the distribution shape of the focal character to estimate natural selection gradients. Avian laying date often presents a skewed distribution and in *manuscript D* we show how selection estimation on such non-Gaussian quantitative character may be biased depending on the genetic and/or environmental origin of the distribution skewness. We then explore how such bias in selection estimation may be involved in the paradox of stasis, of which the laying date is a classic example. *Manuscript E* highlights methods to estimate annual natural selection on a reaction norm and decomposes the selection in selective advantages and selective costs of plasticity. It then illus-

trates how selection on a heritable laying date reaction norm may potentially lead to genetic changes through time. These two *manuscripts D* and *E* show that estimating natural selection on a phenological trait such as laying date in natural environments can be a complex task, and propose simple methods to cope with some of the methodological issues.

2. The challenges to study plasticity in the wild

The study of natural populations is necessary to encompass the huge complexity of ecosystems and its influence on ecological and evolutionary processes. In particular, the study of phenotypic plasticity and its evolutionary potential, to understand how well species can be adapted and/or can adapt to current global changes, requires knowledge on how the environmental variability influences plastic changes. Apprehending the environmental variability experienced by organisms in nature requires to go in nature to estimate environmental heterogeneity. In addition, heritability, that is the relative importance of the additive genetic component over environmental influences (Lynch and Walsh, 1998) on a quantitative trait, can also vary according to the environment where it is estimated (Kruuk et al., 2014; Postma, 2014). Hence, estimating heritability in the laboratory, where the environment is drastically simplified in comparison to nature, often provides irrelevant estimations for the evolutionary potential of populations in the wild. The same is true regarding the estimation of covariance between phenotypic traits, such as covariance between traits in different environmental conditions (Gienapp et al., 2017). Also, estimating heritability and phenotypic plasticity to understand the living world should be done in nature, where natural selection is acting (Kruuk et al., 2014; Gienapp et al., 2017). However, the fact that we do not control and/or take into account all the dimensions of the natural environment can lead to some issues in the study of – among others – the laying date plasticity.

The need for (very) long-term monitoring. Estimation of parameters of interests to understand eco-evolutionary processes such as relationships across trophic levels (e.g. blue tits and caterpillars), phenotypic plasticity, heritability and natural selection requires very large data sets, especially in natural populations, where the uncontrolled environmental heterogeneity scrambles the signals (Clutton-Brock and Sheldon, 2010) and slows down investigations in new study species.

An uncontrolled environment. In nature, because environmental variation cannot be totally described and/or taken into account in models, estimation and interpretation of eco-evolutionary parameters can often be biased due to uncontrolled covariance between phenotype and environment. One of the most difficult issues related to the uncontrolled phenotype – environment covariation is probably for the estimation of natural selection. Indeed, environment can

influence both phenotype and fitness of individuals, inducing environmentally-induced covariance between trait and fitness, and so leading to overestimation of the selection (Rausher, 1992; Kruuk et al., 2003). This may have huge impacts in the estimation of predicted response to selection when estimated using the ‘breeder’s equation’ based on selection gradient estimation (Lande and Arnold, 1983) and heritability of the traits (Morrissey et al., 2010). Such issues can be dealt with using the Robertson-Price identity to predict evolution because it directly estimate the genetic correlation between fitness and phenotype, that is the predicted response to selection (Frank, 2012). Also, there could be environmental coupling between heritability and selection, leading to unreliable predictions of response to selection because the environmental influence is generally not (or cannot be) take into account (e.g Wilson et al., 2006). However, the recent meta-analysis of Ramakers et al. (2018) shows poor evidence for this environmental coupling in natural populations.

A multidimensional environment. The environment can influence phenotypic expression in a complex way since many environmental factors can influence the phenotype. The several environmental factors influencing the phenotypic expression can have additive effects and/or can interact with each other, producing complex links between environmental conditions and the expressed phenotypes. If the number of environmental factors is high, even if each factor has small or moderate influence on the phenotype, different environmental conditions may lead to different phenotypes due to a multi-dimensional plastic response. This can be an issue in the study of phenotypic plasticity because if many factors drive the phenotypic expression, detecting the main environmental drivers may be statistically challenging because of low statistical power, and the description of reaction norm is complicated in several dimensions.

The need for complementary experimental studies. Because most of the studies in natural populations are based on correlations from which causality cannot be inferred, in many cases the relationship between environment, phenotype and/or fitness in natural conditions benefits from complementary experimental studies in controlled environments. As an example, for the identification of environmental drivers of the phenotypic variation through plasticity (i.e. identification of the cue), experimental designs allowing to test for the causal effects of an environmental factor on the phenotype allow to decipher the causal effects of each driver. As a conclusion, the study of phenotypic plasticity and other ecological and evolutionary processes should be studied using the complementary approaches of the studies in natura and in the laboratory (when possible), allowing both to encompass the environmental variability of the environment and to test for causality.

3. Going further

In this part, I will share some personal ideas about interesting questions that remain to explore in the study of the Mediterranean blue tits.

Blue tit phenology in a trophic chain. Blue tits adjust their reproductive period according to (spring) temperature allowing bird to (more or less, depending on the populations and the years) synchronise their reproductive period with the period of maximal abundance of nutritive resources for nestlings. The main nutritive resources of blue tit nestlings are caterpillars (Blondel et al., 1991), as for other passerines such as great tits. The study of insectivorous passerines reproductive phenology is generally done, at best, in a two trophic level system: the birds and the caterpillars. However, the phenology of the caterpillars is also highly plastic, allowing to follow the host tree phenology of outflow (van Asch and Visser, 2007). Also, the impact of climate change on the different species across a trophic level is not necessarily the same, leading to mismatches between some levels of the trophic chain, which may have important consequences on population dynamics and persistence (Visser and Gienapp, 2019). As an example, the trees, the caterpillars and the blue tits do not necessary used same environment cue(s) to modulate their phenology, and climate change may lead to a cascade deregulation between the phenologies of the three trophic levels. An integrative view including the three relevant trophic levels involved in the phenology of blue tits, as done in great tits from the Netherlands (Salis, 2015) or in English blue tits, great tits and pied flycatchers (Burgess et al., 2018) for example, should allow a better understanding of the eco-evolutionary process involved in our Mediterranean blue tit populations. In our study sites, the phenology of caterpillars is currently followed in three of the four sites, and the oak tree phenology is followed since few years, mainly by Samuel Caro, which should provide useful data for the study of the phenology of the ‘blue tits – caterpillars – oak’ system in a couple of years. Also, in my knowledge, we do not know about the population dynamics of the predators of the birds in the study sites. Because predators can induce selective pressures involved in the evolution of phenological strategies, if feasible, it may be very interesting to add this fourth level of the trophic chain in future monitoring and analyses.

Transgenerational plasticity. Transgenerational plasticity is the expression of offspring phenotypes influenced by parental environment (Donelson et al., 2018). It should be favourable when the environment is variable in time and the environmental conditions experienced by the parental generation are correlated to the environment of the offspring (Leimar and McNamara, 2015). Empirical studies have shown that transgenerational plasticity can be adaptive in nature, as for example in the American bellflower (*Campanula Americana*) where life-history strategy of individuals is adaptively influenced by the light exposition of their parents (Galloway and Etterson, 2007). These intergenerational effects of the environment can also be involved in climate adaptation

and climate change, as shown in many experimental studies (see Donelson et al., 2018 for a recent meta-analysis). We know very little (or perhaps even nothing?) about transgenerational plasticity of phenology in birds, and the study of plasticity of laying date both within and across generations could be an interesting – but challenging – future investigation, since transgenerational plasticity may buffer the impact of climate change on population, as it has been shown in marine sticklebacks (Shama et al., 2014) or a tropical reef fish (Donelson et al., 2012) for example.

What about second clutches? Blue tits can lay a second clutch during the season, even though in our study populations, this behaviour is very rare (less than 1% of pairs every year). Despite multiple breeding in a common strategy in passerine populations (e.g. in great tit Husby et al., 2009), in virtually all studies of laying date and reproductive success in blue tits (and more generally in passerines I think), the second clutch is excluded from the analyses because it is considered as another trait than the first clutch of the year (e.g. Husby et al., 2010; Porlier et al., 2012). However, laying a second clutch may influence the reproductive success of individuals (Husby et al., 2009). Females may lay a new clutch when the first one has failed because of predation for example (i.e. ‘replacement clutch’). Also, we could imagine a strategy ‘to spread the risk’, where laying two clutches could help to maximize chances to succeed when taking into account both the phenology of caterpillar preys and the intraspecific competition for resources around the caterpillar peak date of abundance. Such influence of population density and nutritive resource availability on the reproductive success have been shown for example in red squirrels (Descamps et al., 2009). Also, there can be negative correlation between body condition and the number of clutches laid by females either because of egg laying requires resources and decreases body condition of females, or because birds that lay a second clutch may be the females that failed their first clutch of the season, plausibly because of poor body condition. Jarle Tufto is currently studying theoretical implications of taking into account the first and the second clutches in selection estimation models. Depending on the strategy, we can supposed that laying only one or two clutches lead to changes in the optimal laying date, as well as the adaptiveness of the laying reaction norm with spring temperature. It could be very interesting to study the prevalence of the two strategies among populations across a large spatial scale, in Europe as an example, to assess how this strategy could be linked to environmental conditions. In addition, climate change has been shown to be related to decreasing probability of double brooding in great tits and blue tits (Husby et al., 2009; Matthysen et al., 2011), it could thus be interesting to compare effects of climate change in the different populations depending on local environmental conditions.

Extreme events and plasticity. Climate change is a global increase in temperature but also an increase in the rate and/or intensity of extreme climatic events. Extreme temperature events may influence natural selection on laying date (Marrot et al., 2017) and its phenological variation (Bonamour et al.,

2019) in blue tits. Extreme events can also impact population dynamics, as in Albatross for example, where the extreme sea surface temperature influence demography (Pardo et al., 2017). Extreme climatic events can also represent drastic mortality events, maybe threatening population persistence in the long run, as in the shorebird *Haematopus ostralegus* where extreme flooding events can be lethal for chicks in the lowest nests (Bailey et al., 2017). Also, because extreme climatic events are often considered as unpredictable (but this is not necessary the case), adaptive phenotypic plasticity may not be able to evolve in such unpredictable environmental (Bailey and van de Pol, 2016). Extreme climatic events should thus be more generally taken into account in the studied of phenological variation and the impact of climate change on natural populations.

Mixing approaches and methods. Quantitative genetics and the study of phenotypic plasticity in wild populations may benefit from genomics approaches (Gienapp et al., 2017). One of the main advances may be the possibility to estimate a genomic relationship matrix to play the role of pedigree in animal models, for estimation of additive genetic variance (Kruuk, 2004). Such genomic pedigree provides realised genetic similarities among all individuals studied in the population (without assumption on the genes transmission probability to the next generation of 0.5), which allows higher statistical power than the classic social pedigree, where genetic relationships are assumed based on familial interactions, and sometimes do not reflect genetic relationships due to extra-pair paternity and other unknown parentages (Gienapp et al., 2017). Also, a genomic pedigree can allow to use the realised genome sharing among individuals to quantify fitness (Gienapp et al., 2019), which may provide a better estimation than the number of fledgling or the number of recruits, because it estimate the long-term contribution of individuals to future generations (in the same line as Reid et al., 2019).

Also, it seems to me that quantitative genetics in wild populations and demography studies have often same general purpose, that is to study adaptation and its consequences for population dynamics and persistence. For example, in the quantitative genetic studies in wild population, conclusion are often toward the plausible consequences for population dynamics and persistence, but this is not very often properly tested. Also, natural selection is more often estimated using fecundity proxies, and more rarely based on survival, while both fecundity and survival influence population dynamics (but see e.g. the ‘Aster model’, Shaw et al., 2008; Shaw and Geyer, 2010). However, I have the feeling that the two domains do not exchange enough, at least regarding dataset exchanges, which is probably a shame because data needed and available for these two approaches are often the same, that is trait quantifying fecundity and survival, with individual monitoring. From the point of view of a quantitative biologist, I guess there are many long-term data that are unfortunately only studies in a population dynamic perspectives, and in monitoring where the individuals were blood sampled, we may be able to estimate a genomic pedigree to do quantitative genetics. Exchanges among the two disciplines may allow

to more often include evolutionary potential and evolutionary predictions in population dynamic models to predict population persistence in the long run. Hence, more exchanges among these two biological domains could be beneficial to each other, as data are available for both but not necessary the skills.

4. Concluding remarks

All ecosystems in the planet are currently changing because of anthropogenic activities (Palumbi, 2001; Walther et al., 2002; Parmesan and Yohe, 2003; Parmesan, 2006). At the moment, phenotypic plasticity is one of the main response to climate change allowing organisms to cope with rapid environmental change (e.g. Gienapp et al., 2007; Merilä and Hendry, 2014). However, the plastic response most likely will not be sufficient to cope with the current pace of environmental change (e.g. Radchuk et al., 2019) and microevolution of traits under natural selection will be necessary to allow population persistence in the long run. Studies of quantitative genetics, and phenotypic plasticity of phenological traits in natural populations are dominated by vertebrates, mainly bird and mammals species (despite increasing number of other taxa in the literature Kruuk et al., 2014). What about the plasticity and evolutionary potential of snails, mosses or fungus for example? I think we really need to diversify studies towards ‘unloved species’ – often being key levels of the trophic chains in ecosystems – to really understand the impact of global and climate change on nature, as a whole, and maybe be able to cope with it.

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Work with friends.

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Résumé de la thèse en français

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Les organismes vivent dans des environnements qui varient dans l'espace et le dans temps. Les espèces peuvent s'adapter à la variabilité environnementale par différents mécanismes (non exclusifs), selon les échelles temporelles et/ou spatiales et la prévisibilité des variations environnementales (Botero et al., 2015; Tufto, 2015). Premièrement, les organismes peuvent s'adapter aux changements environnementaux par le biais de l'évolution génétique adaptative, c'est-à-dire la modification de la fréquence des allèles dans le temps causée par la sélection naturelle. Une telle microévolution peut se produire dans des environnements hétérogènes à large échelle, c'est-à-dire lorsque l'ampleur de la variation environnementale est beaucoup plus grande que celle rencontrée par les individus au cours d'une génération, indépendamment de la prévisibilité environnementale. Par exemple, chez les guppies (*Poecilia reticulata*), les mâles de différentes populations naturelles de Trinidad diffèrent par leur couleur, leur morphologie et leur comportement. Il a été démontré que ces différences résultent de l'adaptation locale à différentes communautés de prédateurs par différenciation génétique (Reznick, 1982; Reznick et al., 1997).

Deuxièmement, lorsque l'échelle temporelle et/ou spatiale des variations environnementales est de l'ordre d'une génération et que les changements environnementaux sont imprévisibles, une stratégie de répartition des risques est avantageuse (Botero et al., 2015; Tufto, 2015). Un génotype ayant une stratégie de 'bet-hedging' produit des descendants de phénotypes diversifiés, adaptés aux différentes conditions environnementales. Ainsi, dans n'importe quel environnement, certains descendants seront capables de survivre et de se reproduire parmi la progéniture d'un tel génotype (voir par exemple Starrfelt and Kokko, 2012; Childs et al., 2010). L'un des exemples les plus connus de 'bet-hedging' est l'adaptation des plantes aux précipitations imprévisibles, où les graines ont des temps de dormance variables (Cohen, 1966).

Enfin, lorsque la variabilité environnementale est prévisible et que les échelles temporelles (et/ou spatiales) de la variation environnementale sont de l'ordre d'une génération (ou des capacités de dispersion des individus) ou moins, la plasticité phénotypique est sélectionnée (Botero et al., 2015; Tufto, 2015).

1. La plasticité phénotypique

La plasticité phénotypique est l'expression, par un génotype de différent phénotype en fonction de l'environnement (Pigliucci, 2001; DeWitt and Scheiner, 2004; Streans, 1989). Le concept de plasticité phénotypique a une longue histoire scientifique (Woltereck, 1909), mais connaît un intérêt renouvelé dans la communauté scientifique ces dernières décennies. La plasticité est omniprésente dans la nature (Travis, 1994; Pigliucci, 2001; DeWitt and Scheiner, 2004) et englobe une large gamme de phénomènes biologiques tels que : i) différents patrons de coloration des ailes en fonction de la saison de développement chez le 'map butterfly' *Araschnia Levana* (Friberg and Karlsson, 2010) ; ii) la différenciation des castes ouvrières selon les conditions d'élevage chez la fourmi *Pheidologeton diversus* (Holldobler and Wilson, 1990)

; iii) l'assombrissement de la peau chez l'homme en fonction de l'exposition au soleil ou iv) le changement de couleur, de forme et de comportement de la pieuvre *cyanea mollusca* selon l'environnement local (Josef et al., 2012). Alors que les deux premiers exemples sont de la plasticité développementale, c'est-à-dire le changement des voies de développement en fonction de l'environnement, conduisant à des phénotypes différents et irréversibles, les deux derniers exemples sont des cas de plasticité labile. La plasticité labile est le changement réversible d'un phénotype en fonction de l'environnement (c'est-à-dire que le phénotype suit les changements environnementaux plusieurs fois au cours de la vie d'un individu). Lorsque l'échelle temporelle de la variation environnementale est proche du temps de génération, la plasticité phénotypique développementale irréversible est favorisée par la sélection naturelle. Au contraire, la variation environnementale à une échelle plus courte que le temps de génération favorisera l'évolution de la plasticité labile réversible (Botero et al., 2015; Tufto, 2015; Gabriel et al., 2005).

La plasticité phénotypique peut évoluer lorsque la sélection naturelle d'un caractère varie en fonction de conditions environnementales prédictibles. L'environnement agit alors comme un indice qui fournit des informations sur les pressions sélectives futures sur le phénotype, permettant aux organismes de moduler leur phénotype de manière à correspondre au phénotype favorisé par la sélection naturelle dans les conditions environnementales futures.

L'un des exemples les plus célèbres de plasticité phénotypique est la réponse à la présence de prédateurs chez certaines espèces de Daphnies. Par exemple, chez *Daphnia lumholtzi*, un génotype peut produire des individus avec une tête ronde et une petite queue en l'absence de prédateur, et des individus avec un casque à pointe et une longue queue en cas d'exposition à des indices chimiques (ou kairomones) indiquant la présence de prédateurs (voir par exemple Agrawal, 2001). Cela s'explique par le fait que la probabilité que des prédateurs soient présents est plus élevée lorsque des kairomones sont détectés que lorsqu'ils ne le sont pas ; et le phénotype à casque confère une meilleure survie que le phénotype à tête ronde en présence de prédateurs car il protège des attaques des prédateurs (cependant, le phénotype à casque est probablement coûteux en l'absence de prédateur). Ainsi, la production du phénotype à casque lorsque des indices chimiques de prédateurs sont détectés dans l'environnement devrait procurer une meilleure valeur sélective que la production du phénotype à tête ronde, et réciproquement. Par conséquent, la plasticité de la forme du corps en présence de prédateurs chez *Daphnia lumholtzi* a été sélectionnée.

2. Le changement global et la plasticité phénotypique

Les activités humaines provoquent des changements environnementaux à l'échelle planétaire. Ces changements sont si importants que les scientifiques définissent notre époque comme l'anthropocène à l'échelle géologique (voir par

exemple Lewis and Maslin, 2015). Les organismes vivants connaissent actuellement des bouleversements globaux tels que l'urbanisation, la pollution et/ou le changement climatique. On s'attend à ce que la quasi-totalité de ces changements de l'environnement à grande échelle modifient les pressions de sélection, entraînant l'évolution des populations naturelles (Vitousek et al., 1997; Alonso et al., 2001; Palumbi, 2001; Laporte et al., 2016; Pigeon et al., 2016; Fugère and Hendry, 2018). Les nouvelles conditions environnementales peuvent être différentes de celles de l'environnement où les espèces ont évolué à long terme, et la relation entre les espèces peut être modifiée, menaçant la persistance des populations (Radchuk et al., 2019; Visser and Gienapp, 2019). Parmi les différentes composantes du changement global, le changement climatique a un impact important sur les populations naturelles du monde entier (Walther et al., 2002; Parmesan and Yohe, 2003; Parmesan, 2006)). Plusieurs méta-analyses montrent que les organismes ont généralement des phénologie plus précoces en raison du réchauffement climatique, surtout en ce qui concerne les événements du cycle biologique printanier (Walther et al., 2002; Parmesan and Yohe, 2003; Radchuk et al., 2019). La plasticité phénotypique est l'un des mécanismes les plus importants permettant la persistance des populations dans le contexte du changement climatique (Matesanz et al., 2010; Gienapp and Brommer, 2014; Merilä and Hendry, 2014; van Baaren and Candolin, 2018). C'est particulièrement vrai pour les caractères phénologiques qui conditionnent la valeur sélective des individus et qui sont souvent héréditaires et fortement liés au climat, particulièrement dans les régions saisonnières (Charmanier et al., 2014).

La plasticité phénotypique pourrait ne plus être adaptative dans les conditions environnementales nouvelles et/ou extrêmes créées par le changement global rapide. Les changements planétaires peuvent modifier la fiabilité des indices environnementaux en modifiant leur corrélation avec l'environnement sélectif, ce qui conduirait à une diminution de l'avantage adaptatif de la plasticité et sélectionnerait pour une plasticité plus faible (Gavrilets and Scheiner, 1993). Lorsque cela se produit, l'indice environnemental lui-même (par exemple la température, les précipitations ou leur combinaison) ou la fenêtre temporelle où il est évalué peut évoluer. De plus, les organismes peuvent être soumis à de nouvelles conditions environnementales qui n'ont pas été rencontrées au cours de l'évolution de l'espèce, de sorte que les réponses plastiques pourraient produire un phénotype non adapté dans le nouveau milieu (Ghalambor et al., 2007; Chevin and Hoffmann, 2017). Enfin, même si la plasticité phénotypique est encore adaptative dans le contexte du changement global, la norme de réaction peut ne pas permettre de produire le phénotype optimal en raison de variations plastiques trop faibles en réponse à l'environnement. Dans ce cas, la norme de réaction sera sélectionnée pour une plus grande plasticité. Une méta-analyse récente de Radchuk et de ses collaborateurs sur des populations naturelles de vertébrés a montré que les réponses adaptatives au changement climatique (médiées par la micro-évolution et/ou la plasticité phénotypique) sont globalement insuffisantes pour permettre la persistance à long terme de la population (Radchuk et al., 2019). De plus, la plasticité peut

ne pas être assez grande pour limiter l'impact du réchauffement climatique, du moins chez les ectothermes (Gunderson and Stillman, 2015) et les plantes (Duputié et al., 2015).

On s'attend donc à ce que les changements globaux conduisent souvent à une sélection sur la plasticité, avec une direction qui dépend du type de changement environnemental. Les défis scientifiques actuels consistent à évaluer si la plasticité phénotypique est suffisante pour permettre la persistance des populations dans un contexte de changement global et, dans la négative, à comprendre si et comment la plasticité peut évoluer pour aider les populations à faire face à la nouvelle variabilité environnementale anthropogénique.

3. Plan de la thèse

3.1 Les objectives généraux

La phénologie, c'est-à-dire la chronologie des événements du cycle biologique d'une population, est cruciale pour la persistance d'une population, et en particulier la phénologie de la reproduction. La phénologie est généralement plastique avec le climat, ce qui en fait une des caractéristiques des populations les plus touchées par le changement climatique (Parmesan and Yohe, 2003; Parmesan, 2006; Radchuk et al., 2019). L'étude de la plasticité de la phénologie dans les populations sauvages est donc importante pour comprendre les impacts du changement climatique sur les caractéristiques et la persistance des populations.

Bien qu'ils ne représentent qu'environ 0,0004% de la biomasse totale de la planète (environ 0,002 Giga tonnes de carbone, Bar-On et al., 2018), les oiseaux sont présents partout dans le monde, dans tous les écosystèmes et sont étudiés depuis longtemps par les ornithologues, ce qui fournit une connaissance écologique et biologique exceptionnelle sur ce taxon. Actuellement, les nombreux suivis à long terme de populations d'oiseaux sauvages permettent de montrer que les oiseaux sont fortement affectés par le changement climatique (Møller et al., 2019).

La date de ponte chez les oiseaux, en particulier chez les passereaux, est un exemple typique de la phénologie de la reproduction dans la nature. La date de ponte est généralement héritable (Postma, 2014) et plastique en réponse aux conditions environnementales printanières, et la variation de la date de ponte d'une année à l'autre est particulièrement bien corrélée aux variations de température au début du printemps dans les régions tempérées (par exemple Brommer and Rattiste, 2008; Phillimore et al., 2016). Le moment de la reproduction est aussi généralement étroitement lié à la valeur sélective des individus, est souvent soumis à une sélection directionnelle négative pour une reproduction précoce (Porlier et al., 2012) et peut évoluer génétiquement (par exemple Verhagen et al., 2019). Nous savons que la sélection de la date de ponte peut être liée aux conditions climatiques moyennes ainsi qu'aux événements extrêmes, qui sont de plus en plus fréquents avec le changement climatique dans les populations de mésanges bleues méditerranéennes notamment (Marrot

et al., 2017, 2018). En plus de l'influence climatique, le succès de reproduction annuel des oiseaux dépend de la synchronisation entre leur période de reproduction et la période de développement de la principale ressource nutritive des poussins, les chenilles (Blondel et al., 1991). On s'attend donc à ce que l'écart de temps entre la reproduction des oiseaux et la période de développement des chenilles soient un environnement sélectif pour les mésanges bleues dans les régions tempérées (Visser and Gienapp, 2019).

Par conséquent, l'étude à long terme des mésanges bleues en région méditerranéenne offre une occasion exceptionnelle d'étudier la plasticité phénotypique de la phénologie de la reproduction en population naturelle.

Au cours de cette thèse, j'ai étudié la plasticité phénotypique d'un trait phénologique répété, la date de ponte chez la mésange bleue (*Cyanistes caeruleus*), un exemple classique de plasticité en population naturelle. J'ai d'abord étudié comment le(s) indice(s) environnemental(aux) de la phénologie de reproduction varient parmi quatre populations de mésanges bleues méditerranéennes et de leur principale proies, et comment cette variation affecte la fiabilité des indices, notamment dans le contexte du changement climatique. J'ai ensuite exploré la variabilité intra-individuelle de la date de ponte, selon la qualité de l'environnement, l'âge et les températures printanières. Troisièmement, je me suis demandé si l'estimation de la sélection naturelle sur un caractère à distribution asymétrique, comme la date de ponte, peut être biaisée, et comment cela pourrait contribuer à expliquer le paradoxe de la stase. Enfin, j'ai étudié le potentiel évolutif de la plasticité de la date de ponte en fonction de la température, en estimant sa variabilité inter-individuelle, son héritabilité et la sélection naturelle à laquelle elle est soumise.

L'étude de la plasticité phénotypique en laboratoire est sans aucun doute essentielle et méthodologiquement plus facile que dans les populations sauvages. Néanmoins, la prise en compte de l'ensemble des effets environnementaux sur la variation phénotypique des populations naturelles est nécessaire pour comprendre les processus écologiques et ainsi peut être pouvoir prédire les trajectoires évolutives, et cela ne peut se faire que dans des conditions naturelles pertinentes où vivent les populations sauvages. Il en va de même pour le potentiel évolutif estimé par la variance génétique additive, la sélection naturelle et la réponse à la sélection. Comme c'est souvent le cas dans les études de génétique quantitative les populations sauvages (Clutton-Brock and Sheldon, 2010), l'étude de la plasticité phénotypique et de son potentiel évolutif exige un important jeu de données comportant plusieurs observations phénotypiques sur les mêmes individus (Martin and Festa-Bianchet, 2011; van de Pol, 2012), résultant généralement d'un suivi longitudinal à long terme sur le terrain.

3.2 Le suivi long-terme des mésanges bleues méditerranéennes

Sites d'étude

L'étude de la mésange bleue dans la région méditerranéenne française offre une occasion rare d'explorer la plasticité phénotypique dans un environnement naturel. Les mésanges bleues (*Cyanistes caeruleus*) sont des passereaux communs dans le Paléarctique occidental, qui habitent un large éventail d'écosystème tels que les forêts, les zones agricoles, les jardins et les parcs, à la campagne comme dans les villes. Un suivi à long terme des populations de mésanges bleues dans le sud de la France a été initié par Jacques Blondel en 1976. Nous nous concentrons dans cette thèse sur quatre populations de mésanges bleues dans cette région : les populations de D-Rouvière (près de Montpellier en France métropolitaine, suivies depuis 1991) et de D-Muro (en Corse, suivies depuis 1993) se situent dans des forêts dominées par le chêne pubescent à feuilles caduques (*Quercus pubescens* ; 'Deciduous' en anglais, d'où le "D"), tandis que les populations de E-Pirio et de E-Muro (en Corse, suivies respectivement depuis 1976 et 1998) se situent dans des forêts à chêne vert à feuilles persistantes (*Quercus ilex* ; 'Evergreen' en anglais, d'où le "E"). Les deux types d'écosystèmes forestiers influencent les traits d'histoire de vie des mésanges bleues et les oiseaux sont localement adaptés à chaque habitat (Blondel et al., 2006; Charmantier et al., 2016).

La saison de reproduction des mésanges bleues

Chaque printemps, les femelles et les mâles construisent un nid (principalement avec de la mousse dans la forêt), et lorsque le nid est terminé, les femelles pondent leurs oeufs. La taille de de la nichée varie généralement de 5 à 15 œufs selon les conditions environnementales et la population. Après la ponte de toute la couvée, les femelles incubent les œufs pendant 14 jours. De l'éclosion des poussins jusqu'à ce qu'ils s'envolent hors du nid et prennent leur indépendance, les parents les nourrissent principalement de chenilles, mais aussi d'autres proies telles que des araignées (Blondel et al., 1991). Les estimations montrent qu'un poussin a besoin d'être nourri d'environ 1800 chenilles pendant son développement (Blondel et al., 1991; Tremblay et al., 2005). Les jeunes oiseaux ont les besoins nutritifs les plus importants lorsqu'ils atteignent environ 9 jours. La synchronisation entre la date d'abondance maximale de la chenille et le développement du poussin devrait donc être cruciale pour la condition des poussins et le succès de reproduction de leurs parents (e.g. Visser et al., 2006).

Collecte de données

Pendant la saison de reproduction, chaque couple d'oiseaux qui se reproduisent dans les nichoirs aménagés dans les forêts fait l'objet d'un suivi. Il est à noter que les oiseaux vivent en liberté, mais que les nichoirs sont en

béton fabriqué par l'homme, ce qui peut affecter les phénotypes des oiseaux, bien que ce biais potentiel ne puisse être estimé dans notre plan expérimental (Møller, 1992). De plus, la densité de population augmente avec le nombre de nichoirs fournis dans la forêt, ce qui devrait avoir des impacts sur la dynamique des populations. Cependant, nous ne savons pas quelle était la densité de population naturelle des mésanges bleues dans les forêt étudiées avant le début du suivi. Ces populations de mésanges bleues peuvent donc être qualifiées de populations semi-sauvages. Chaque semaine, tous les nichoirs sont contrôlés pour déterminer la date de ponte, le nombre d'œufs pondus et le nombre d'oisillons qui s'envolent le nid pour chaque tentative de reproduction. En plus des données sur le succès phénologique et reproductif, les mesures morphologiques habituelles chez les oiseaux, comme la longueur du tarse et de l'aile, ainsi que des échantillons de sang sont recueillis pour chaque oiseaux se reproduisant dans nos nichoirs. Les poussins sont bagués au nid avec un numéro unique, tout comme les parents lorsqu'ils les nourrissent. Le suivi permet également de produire un pedigree social pour des analyses de génétique quantitative. Des études antérieures de Charmantier et de ses collaborateurs ont montré un taux de paternité hors couple élevé dans nos populations (18-25% en Corse, Charmantier et al., 2004, qui est positivement corrélé à la densité de population, Charmantier and Perret, 2004). Les héritabilités sont donc sous-estimées dans nos populations d'étude puisqu'elles sont basées sur des pedigrees sociaux plutôt que génétiques, mais cette sous-estimation devrait être raisonnable (moins de 15%, Charmantier and Réale, 2005; Firth et al., 2015, mais voir Bourret and Garant, 2017). En plus du suivi des mésanges bleues, nous avons recueilli des données sur la phénologie des chenilles. La masse d'excréments de chenilles est prélevée à l'aide de copromètres (carré de 50cm*50cm avec un collecteur d'excréments) pendant la saison de reproduction des oiseaux, permettant d'estimer l'abondance des chenilles. La date d'abondance maximale des chenilles est le jour où la quantité d'excréments récoltés est la plus élevée.

Sélection naturelle

Le principal indice environnemental de plasticité de la date de ponte est la température au début du printemps pour les mésanges bleues (par exemple Porlier et al., 2012, comme pour les passereaux des régions tempérées en général, Visser et al., 2009; Caro et al., 2013). La date de ponte est sous sélection directionnelle négative dans les quatre populations étudiées (Porlier et al., 2012; Marrot et al., 2017, 2018), ce qui signifie que les individus précoces ont un succès de reproduction annuel plus élevé que les individus tardifs (comme c'est le cas généralement chez les autres passereaux et oiseaux). On s'attend à ce que la date de ponte soit sous sélection stabilisante (comme chez les mésanges charbonnières, par exemple Chevin et al., 2015), et une sélection quadratique négative a été détectée dans la population E-Pirio (de Villemereuil, *en préparation*), ce qui suggère qu'il existe une date de ponte optimale associée à un succès reproducteur annuel maximal. En raison du lien entre la phénologie de la reproduction des oiseaux et la date du pic d'abondance

des chenilles (qui est également plastique en ce qui concerne les variations environnementales, par exemple van Asch and Visser, 2007), le degré de synchronisation entre les oiseaux et les chenilles devrait être un facteur clé de sélection sur la date de ponte, c'est-à-dire une des dimensions de l'environnement de sélection (Reed et al., 2013a,b; Ramakers et al., 2018; Visser and Gienapp, 2019).

3.3 Feuille de route

Au cours de cette thèse j'ai étudié la variabilité, l'héritabilité et la sélection naturelle sur la plasticité phénotypique de la date de ponte dans les populations naturelles de mésanges bleues. Alors que le chapitre un traite de la comparaison des quatre populations de mésanges bleues étudiées en Méditerranée, les chapitres suivants se concentrent uniquement sur la population ayant bénéficiée du plus long suivis, la population E-Pirio. Au **chapitre un** (*manuscrit A*), j'examine d'abord l'impact du changement planétaire sur la variation et la fiabilité des indices environnementaux (c'est-à-dire la capacité de prédire l'environnement sélectif futur) dans les populations naturelles. Je détaille ensuite une étude de cas sur la variabilité des indices environnementaux parmi quatre populations de mésanges bleues et deux populations de chenilles, et j'estime la fiabilité des indices de mésanges bleues dans le contexte du changement climatique. Dans le **chapitre deux**, j'étudie la variation individuelle de la plasticité de la date de ponte au cours de la vie dans deux manuscrits. Dans le *manuscrit B*, j'explore la variation avec l'âge de la plasticité de la date de ponte et le succès de reproduction annuel, à la recherche de sénescence de la plasticité. Dans le *manuscrit C*, je me demande si la qualité de l'environnement vécue par les femelles au cours de leur vie affecte leurs trajectoires de sénescence, en utilisant le concept d'âge environnemental. C'est ce qu'on pourrait appeler de la plasticité de la sénescence. Le **chapitre trois** traite de la sélection de la date de ponte et de sa plasticité, avec deux manuscrits. Dans le *manuscrit D*, je propose des développements théoriques et méthodologiques sur la mesure de la sélection et de la réponse à la sélection sur des traits aux distributions asymétriques, comme la date de ponte, et discute des implications pour comprendre le paradoxe de la stase. Dans le dernier *manuscrit E*, j'examine comment la sélection naturelle et la réponse génétique à la sélection de l'élévation et de la pente (plasticité) de la norme de réaction pourraient contribuer au suivi des fluctuations environnementales dans la population E-Pirio. Enfin, je discute des résultats de cette thèse dans une discussion générale et propose des orientations futures pour la recherche.

3.4 Résumés des manuscrits

Manuscrit A

La plasticité phénotypique est un mécanisme majeur de réponse au changement global. Cependant, les réponses plastiques actuelles ne resteront adaptatives dans les conditions environnementales futures que si des indices environnementaux informatifs sont encore disponibles. Nous résumons brièvement

les connaissances actuelles sur l'origine évolutive et les fondements mécanistes des indices environnementaux de la plasticité phénotypique, avant de mettre en lumière les effets potentiellement complexes du changement global sur la disponibilité et la fiabilité des indices. Nous illustrons ensuite certains de ces aspects par une étude de cas comparant la plasticité de la phénologie de la reproduction des mésanges bleues dans deux habitats contrastés : des forêts à feuillages persistants et des forêts à feuilles caduques. À l'aide de jeux de données à long terme, nous étudions les facteurs climatiques liés à la phénologie de reproduction des oiseaux et à leur principale source de nourriture. Les mésanges bleues occupant différents habitats diffèrent considérablement par les indices qui influent sur la plasticité de la date de ponte, ainsi que par la fiabilité de ces indices en tant que prédicteurs du facteur (supposé) de la sélection naturelle, la date du pic chenille. La tendance temporelle de la date de ponte précoce, détectée seulement dans les populations à feuillages persistants, s'explique par l'augmentation de la température au cours de la fenêtre temporelle de leur indice. Nos résultats soulignent l'importance d'intégrer les mécanismes écologiques qui façonnent la variation de la plasticité si nous voulons comprendre comment le changement global affectera la plasticité et ses conséquences sur la biologie des populations.

Manuscrit B

Les traits d'histoire de vie sont souvent plastiques en réponse à des facteurs environnementaux extrinsèques (comme la température ou les précipitations) et à des facteurs internes comme l'âge chez de nombreuses espèces. La variation des caractères au cours de la vie des individus pourrait donc être en partie due à la plasticité de ces caractères en fonction de l'âge. Nous étudions si la plasticité d'un caractère phénologique – la date de ponte en fonction de la température printanière – varie avec l'âge et explorons ses conséquences sur le succès de reproduction annuel des femelles. Nous utilisons les données d'un suivi à long terme d'une population sauvage de mésanges bleues en Corse pour estimer les variations de plasticité de la phénologie de la reproduction et du succès de reproduction annuel en fonction de l'âge. Nous montrons que la plasticité de la date de ponte et le succès de reproduction annuel varient selon l'âge : les femelles jeunes et âgées sont moins plastiques et ont moins de petits que les femelles d'âge moyen. La variation de la plasticité en fonction de l'âge peut avoir des conséquences différentes sur le succès de reproduction au cours de la vie des femelles, car les femelles plus âgées peuvent avoir un succès de reproduction inférieur, quelle que soit leur date de ponte. La plasticité phénologique est un facteur majeur d'adaptation aux variations rapides de l'environnement, nos résultats suggèrent que la compréhension de son rôle dans l'adaptation au changement climatique et la persistance de la population pourrait nécessiter l'intégration de la structure en âge des populations.

Manuscrit C

La sénescence (ou vieillissement), c'est-à-dire la détérioration des performances avec l'âge, est omniprésente dans la nature et très variable parmi les espèces, au sein des populations et parmi les individus dans les populations naturelles. Nous ne savons pas très bien comment les conditions environnementales peuvent influencer sur les trajectoires de sénescence au sein d'une population et si l'âge chronologique seul est suffisant pour décrire le processus de vieillissement dans la nature. Nous explorons donc l'effet de la qualité de l'environnement sur la sénescence de la reproduction et de la date de ponte dans une population naturelle de mésanges bleues. Nous démêlons les effets de l'âge chronologique et de la qualité de l'environnement en introduisant deux estimations complémentaires de l'âge environnemental : le nombre annuel moyen cumulatif de jeunes poussins par couvée et la probabilité de survie annuelle des adultes. Les résultats montrent que les femelles augmentent leur succès reproductif annuel et pondent plus tôt avec l'âge jusqu'à l'âge de 3 ans environ, puis souffrent de sénescence reproductive (diminution du succès reproductif annuel et date de ponte tardive) en vieillissant. Ces trajectoires de sénescence sont influencés par l'âge environnemental : les femelles qui ont connu de meilleures conditions environnementales par le passé présentent une plus forte sénescence reproductive et pondent plus tard avec l'âge que les femelles qui ont connu un environnement plus défavorable. Ces résultats sont en accord avec la théorie du soma jetable et du 'live-fast-die-young', soulignant l'importance de la qualité et de la variabilité de l'environnement dans la formation des trajectoires de sénescence des oiseaux en milieu naturel.

Manuscrit D

Les réponses phénotypiques à la sélection observées dans la nature diffèrent souvent des prédictions fondées sur les mesures de la sélection et de la variance génétique. Une hypothèse négligée pour expliquer ce paradoxe de la stase évolutive est qu'une distribution phénotypique asymétrique affecte la sélection naturelle et l'évolution. Nous montrons par modélisation mathématique que, lorsqu'un caractère sélectionné pour un phénotype optimal a une distribution asymétrique, la sélection directionnelle est détectée même à l'équilibre évolutif, où elle ne provoque aucun changement dans le phénotype moyen. Lorsque les effets environnementaux sont distribués de manière asymétrique, le gradient directionnel de Lande et Arnold (1983) est dans la direction opposée à celle de l'asymétrie. En revanche, les valeurs de reproduction asymétriques peuvent déplacer le phénotype moyen de l'optimum, provoquant une sélection directionnelle dans la direction de l'asymétrie. Ces effets peuvent être décomposés à l'aide d'autres estimations de sélection fondées sur des dérivés moyennes de la fitness relative individuelle ou sur des covariances génétiques additives entre la fitness relative et le trait (identité de Robertson-Price). Nous évaluons la validité de ces prédictions à l'aide de simulations d'estimation de sélection sous des échantillons de taille modérée. Les traits phénotypiques écologiquement pertinents pourrait, de manière générale, avoir des distributions asymétriques,

comme nous l'illustrons ici avec la date de ponte des oiseaux – décrite à plusieurs reprises comme étant plus stable évolutivement qu'attendue par la théorie – de sorte que cette asymétrie devrait être prise en compte dans l'étude de la dynamique évolutive en populations naturelles.

Manuscrit E

Les organismes vivent dans des environnements qui varient dans le temps avec une ampleur, une vitesse et une prévisibilité différentes. Les changements climatiques actuels modifient les tendances de ces fluctuations, ce qui pourrait modifier les régimes sélectifs dans les populations naturelles. En particulier, on s'attend à ce que la sélection sur la plasticité phénotypique dépende fortement des modèles de fluctuations environnementales, notamment de la prévisibilité de la sélection par l'indice environnemental induisant une réponse plastique. Ceci affecte à son tour l'évolution de la plasticité, si elle est héréditaire. Cependant, il existe peu de preuves empiriques de la sélection de la plasticité dans les populations naturelles, et des preuves de la fluctuation de la sélection et de la réponse à la sélection de la plasticité sont encore plus rares. A l'aide d'un suivi à long terme de mésanges bleues sauvages méditerranéennes en Corse, nous estimons la sélection annuelle sur la plasticité, ainsi que les coûts potentiels de la plasticité, à partir de mesures individuelles de la valeur sélective et des traits, combinés avec des séries chronologiques environnementales. Nous détectons un environnement fluctuant dans notre site d'étude, et nos résultats semblent indiquer une sélection naturelle variable dans le temps, tant au niveau de la date de ponte que de sa plasticité. Nos résultats préliminaires suggèrent également que l'élévation de la norme de réaction et la pente peuvent toutes deux répondre à une sélection fluctuante au fil des années, et que ces changements sont prédits par l'équation multivariée de l'éleveur pour l'interception, mais pas pour la pente de la norme de réaction.

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Abstract. Phenotypic plasticity – the expression by a single genotype of different phenotypes according to the environment – is ubiquitous in nature. Changes in phenology through plastic modulations are currently one of the main responses to climate change in the wild. Despite allowing adaptive and fast responses to environmental variation in the past, phenotypic plasticity may not be adaptive anymore in the new and/or extreme environmental conditions created by rapid global change. In this context, evolution of phenotypic plasticity could be crucial for population adaptation and persistence. One of the most studied examples of phenological plasticity is the variation of avian egg-laying date according to spring temperature which acts as an environmental cue in most of passerines species in temperate regions. Taking advantage of the long-term monitoring of four wild populations of Mediterranean blue tits (*Cyanistes caeruleus*), this thesis investigates the variability, heritability of and natural selection on phenotypic plasticity of reproductive phenology. First, a review of the influence of climate change on phenotypic plasticity highlighted the importance of potential changes in cue(s) reliability for plasticity to remain adaptive, a point we further explored in our four blue tits populations and their main preys (caterpillars), showing differences among populations from different habitats. Second, to better understand the drivers of within-individual variation of laying date phenology we evaluated how ageing affects plasticity of laying date in response to temperature, and revealed stronger plasticity in mature compared to young or old females. Moreover, we assessed plasticity of reproductive senescence according to the environmental quality experienced by birds during their lifetime and showed that individuals experiencing good environments age faster. Finally, to improve our knowledge of the evolutionary potential of reproductive phenology in this species, we estimated the heritability of laying date and implemented methods to estimate natural selection on laying date and its plasticity. We showed both heritability of and selection on plasticity, we thus expect response to selection.

Key words: Phenotypic plasticity, Phenology, Laying date, Climate change, Cue reliability, Senescence, Natural selection, Response to selection, Blue tits, Wild population

Résumé. La plasticité phénotypique – l’expression par un génotype de différents phénotypes en fonction de l’environnement – est un processus omniprésent dans la nature. Les changements de phénologie sont actuellement l’une des principales réponses au changement climatique. Bien qu’elle ait permis des réponses adaptatives aux variations environnementales par le passé, la plasticité phénotypique pourrait, dans un futur proche, ne plus être adaptative en raison des transformations importantes et rapides des écosystèmes. Dans un tel contexte, l’évolution de la plasticité phénotypique pourrait être cruciale à la persistance des populations, cela nécessitant que la plasticité soit héritable et soumise à la sélection naturelle. Un des exemples les plus étudiés de plasticité phénologique est la variation de la date de ponte en fonction de la température chez les oiseaux. Les températures printanières sont en effet l’indice environnemental le plus corrélé aux changements de phénologie chez la plupart des espèces de passereaux en région tempérée. En se basant sur les suivis à long-terme de quatre populations naturelles de mésanges bleues (*Cyanistes caeruleus*) en région méditerranéenne, cette thèse s’intéresse à la variabilité, l’héritabilité et la sélection naturelle sur la plasticité phénotypique de la phénologie de la reproduction. Premièrement, dans une revue bibliographique sur l’influence du changement climatique sur la plasticité phénotypique, nous avons mis en évidence l’importance des effets probables du changement climatique sur la fiabilité des indices environnementaux. Ce point a fait l’objet d’une étude plus poussée dans nos quatre populations de mésanges ainsi que chez leurs principales proies (chenilles), mettant en évidence des différences entre populations provenant d’habitats différents. Deuxièmement, pour mieux comprendre les déterminants de la variation intra-individuelle de la phénologie de la date de ponte, nous avons exploré comment le processus de vieillissement affecte l’expression de la plasticité phénotypique de la date de ponte en fonction de la température, révélant une plasticité plus forte chez les femelles matures que chez les femelles jeunes ou âgées. De plus, nous avons évalué la plasticité de la sénescence de reproduction en fonction de la qualité environnementale expérimentée par les oiseaux au cours de leur vie, et montré que les individus dans un bon environnement vieillissent plus vite. Enfin, afin de mieux cerner le potentiel évolutif de la phénologie de la reproduction chez la mésange bleue, nous avons estimé l’héritabilité de la plasticité et mis en œuvre de nouvelles méthodes pour estimer la sélection naturelle sur les normes de réaction. Nous avons détecté de l’héritabilité de la plasticité ainsi que de la sélection, nous prédisons donc une réponse à la sélection de la plasticité de la date de ponte.

Mots-clés : Plasticité phénotypique, Phénologie, date de ponte, changement climatique, Fiabilité de l’indice, Sénescence, Sélection naturelle, Réponse à la sélection, mésanges bleues, population sauvage